IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Before the Board of Patent Appeals and Interferences

In re Patent Application of Atty Dkt. 1498-130
C# M#

SQUIRRELL et al. Group Art Unit: 1652

Serial No. 09/529,722 Examiner Steadman

Filed: April 19, 2000 Date August 28, 2003

Title: METHOD FOR OBTAINING LUCIFERASE FREE OF THERMOSENSITIVE ADENYLATE KINASE CATALYTIC ACTIVITY

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

☐ **Correspondence Address Indication Form Attached.**

☐ **NOTICE OF APPEAL**
Applicant hereby appeals to the Board of Appeals from the decision dated _____ of the Examiner twice/finally rejecting claims _____ ($ 320.00) $

☒ An appeal **BRIEF** is attached in triplicate in the pending appeal of the above-identified application ($ 320.00) $ 320.00

☐ Credit for fees paid in prior appeal without decision on merits -$ ( )

☐ A reply brief is attached in triplicate under Rule 193(b) **(no fee)**

☒ Petition is hereby made to extend the current due date so as to cover the filing date of this paper and attachment(s) (**$110.00/1 month**; $410.00/2 months; **$930.00/3 months**; $1450.00/4 months) $ 110.00

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☐ Applicant claims "Small entity" status, enter ½ of subtotal and subtract -$( )
☐ "Small entity" statement attached.

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Any future submission requiring an extension of time is hereby stated to include a petition for such time extension. The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our **Account No. 14-1140.** A duplicate copy of this sheet is attached.

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By Atty: B. J. Sadoff, Reg. No. 36,663

Signature: _____

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of

SQUIRRELL et al. Atty. Ref.: 1498-130

Serial No. 09/529,722 Group: 1652

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For: METHOD FOR OBTAINING LUCIFERASE FREE OF THERMOSENSITIVE ADENYLATE KINASE CATALYTIC ACTIVITY

* *

August 28, 2003

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APPEAL BRIEF

Sir:

Appellants hereby appeals the Final Rejection of claims 67-106, in the Office Action dated May 2, 2003 (Paper No. 30), and submits the present Appeal Brief, in triplicate, pursuant to Rule 192.

(1) Real party in interest

The real party in interest is the Secretary of State for Defence, Defence Evaluation and Research Agency, Ivley Road, Hampshire, Great Britain, GU14 0LX, by way of an Assignment from the applicants, recorded in the U.S. Patent and Trademark Office on April 19, 2000, at Reel 010805, Frames 0566-0568. A separate Associate of Power of Attorney in favor of the undersigned is attached separately.

(2) Related appeals and interferences

The appellant, the undersigned, and the assignee are not aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

(3) Status of claims

Claims 67-106 are pending and have been finally rejected. A copy of the pending claims 67-106 is attached as Appendix A.

Originally-filed claims 1-3 and 5-18 were amended in an Amendment filed February 7, 2001, which also canceled claim 4 without prejudice. Amendments filed after the final rejection on July 27, 2001 and August 27, 2001, have been entered. See, the Advisory Action dated September 17, 2001. An Amendment filed October 18, 2001 after final rejection has not been entered. See, the Advisory Action dated November 15, 2001 (Paper No. 15). The Amendment of October 18, 2001, which canceled claims 1-3 and 5-18 and introduced new claims 19-32, was entered upon a filing of a CPA on December 27, 2001.

Claims 19-32 were canceled, without prejudice, and claims 33-46 were added by way of an Amendment filed April 24, 2002. Claims 33-46 were canceled, without prejudice, and claims 47-57 were added in an Amendment after final rejection filed October 2, 2002, which was entered. See, the Advisory Action dated October 29, 2002 (Paper No.22). A Second Amendment Under Rule 116 filed January 2, 2003, which

canceled claims 47-57, without prejudice, and added claims 58-66 were entered. See, the Advisory Action dated January 14, 2003 (Paper No. 27).

Claims 58-66 were canceled, without prejudice, by way of an Amendment filed April 2, 2003, which also added new claims 67-106, which are the subject of the present Appeal. The Amendment of April 2, 2003, was filed with an RCE of the same date.

Claim 67-106 are pending and the subject of the present Appeal. A copy of the pending claims 67-106 is attached as Appendix A.

(4) Status of amendments

No Amendments have been filed since the date of the latest final rejection dated May 2, 2003 (Paper No. 30).

(5) Summary of the invention

The invention of the claims relates to methods of obtaining polypeptides or protein products, such as enzymes, and in particular luciferases, which are substantially free of undesired contaminants, such as other enzymes, and specifically adenylate kinase, usually found in expression products. The presently claimed invention also provides recombinant host cells and methods of using the same to produce the desired polypeptide product.

The presently claimed invention provides, in particular, use of luciferase, such as by recombinant means, with a reduced amount of adenylate kinase. Specifically, as described in the specification, luciferase is advantageously used to detect adenylate kinase. See, page 2, lines 25-32. Adenylate kinase is well-known as an enzyme which

catalyzes the reaction converting ADP to ATP. The amount of adenylate kinase is measured by adding ADP to a reagent mixture whereby the ADP is converted to ATP by any adenylate kinase present in the mixture. The ATP product is then detected using luciferase/luciferin in a well-known system which may be characterized as follows:

$$Mg{+}{+}ADP + ADP \xrightarrow{\text{Adenylate Kinase}} Mg{+}{+}ATP + AMP$$

$$ATP + Luciferin + O_2 \xrightarrow[Mg{+}{+}]{\text{Luciferase}} Oxyluciferin + AMP + PPi + CO_2 + LIGHT$$

The appellants have appreciated that because adenylate kinase is ubiquitous in recombinant cellular systems, such as those used to produce the luciferase reagent, luciferase preparations may contain residual adenylate kinase which will, at a minimum reduce the sensitivity of these assays by increasing the background signal and, more importantly, produce false positive results. See, page 3, lines 1-7 of the present application. Reduction of adenylate kinase in luciferase reagent preparations is advantageous.

The gene encoding adenylate kinase cannot be inactivated in recombinant cellular systems used to produce luciferase reagents because adenylate kinase is an essential enzyme required for recombinant cellular function.

The appellants have now discovered that luciferase reagent preparations with reduced adenylate kinase content may be produced recombinantly and that the

production method may be applied to production of a desired protein with simultaneous production of an undesired essential protein.

Specifically, the presently claimed invention provides, in one embodiment, a method for producing a polypeptide product, such as luciferase, which is substantially free of a specific undesired protein that hinders the use of polypeptide product, such adenylate kinase, wherein the undesired protein has activity that is essential for survival of a host cell or for a viable production process using a host cell. See, independent claim 67; page 2, line 29 to page 3, line 7 of the present application; page 3, lines 26-27 of the present application and originally-filed claim 1. This method of the claimed invention further involves identifying a mutant form of the polypeptide product which has increased tolerance to a particular reaction condition, such as pH or temperature, than a corresponding wild-type polypeptide product. See, page 4, line 9 to page 5, line 9 of the present application. This embodiment of the claimed invention further provides for identifying a mutant form of the undesired protein, such as adenylate kinase, which has decreased tolerance to the reaction condition as compared to a wild-type form of the undesired protein and is denatured under conditions at which the mutant form of the polypeptide product is stable. See, page 4, line 9 to page 5, line 19 of the present application. This embodiment of the claimed invention further provides for transforming a host cell so that it expresses the mutant form of the polypeptide product and the undesired protein only in the mutant form, in culturing the host cell and recovering the desired product. See, page 5, line 21 to page 6, line 6; page 6, lines 8-13; page 6, lines 24-28; page 8, lines 18-27; and originally-filed claim 2, of the originally-filed application.

Independent claim 68, which is similar to independent claim 67, provides a further embodiment of the claimed invention wherein the polypeptide product has an increased thermostability over a corresponding wild-type polypeptide product and the undesired protein has a decreased thermostability as compared to a wild-type form of the undesired protein. The preferential recovery of the desired product is effected through the difference in temperature stability. See, for example, page 6, lines 15-16 of the present application.

The specific temperature recitations of dependent claim 69 are supported by, for example, page 5, lines 6-9 of the present specification.

The specific recitations of dependent claim 70 are supported, for example, by the specification on page 8, lines 7-12.

The specific temperature recitation of dependent claim 71 finds support, for example, in the originally-filed claims 5 and 8 as well as the specification at page 5, lines 6-9.

The specific recitations of dependent claim 72 are supported by originally-filed claim 7 and the specification at page 6, lines 8-13.

The alternative method for producing a polypeptide product of independent claim 73 is described, for example, at page 6, lines 8-13 and page 8, lines 18-24 of the specification as well as the presently filed claims 1 and 15.

The specific recitations of dependent claim 74 are described, for example, in originally-filed claim 2.

The specific recitations of the method of dependent claim 75 finds support, for example, in originally-filed claims 3 and 6.

The specific recitations of dependent claim 76 finds support, for example, in originally-filed claims 3 and 4.

The specific recitations of the embodiment recited in dependent claim 77 find support, for example, in originally-filed claim 5.

The specific recitations of dependent claim 78 finds support, for example, in originally-filed claim 5.

The specific recitations of the embodiment recited in dependent claim 79 find support, for example, in originally-filed claim 6.

The specific recitation of dependent claim 80 finds support, for example, in page 6, lines 8-13 of the specification and originally-filed claim 7.

The specific recitations of the method of dependent claim 81 find support, for example, in page 5, lines 6-9 of the specification as well as originally-filed claims 5 and 8.

The specific recitations of dependent claim 82 find support, for example, in page 5, lines 11-19 of the specification and originally-filed claim 9.

The recombinant cell recited in independent claim 83 is described, for example, at page 6, lines 8-13 and originally-filed claim 10.

The recitation of dependent claim 84 finds support, for example, at page 6, lines 8-13 and originally-filed claim 11.

The recitation of dependent claim 85 finds support, for example, at page 6, lines 18-19 and originally-filed claim 12.

The recitations of dependent claims 86 and 87 find support, for example, at page 6, lines 21-22 and originally filed claim 13.

The recitation of embodiments of the method claimed in dependent claim 88 finds support, for example, in page 6, lines 24-30 of the specification of originally-filed claim 15.

The specific recitations of dependent claim 90 find support, for example, at page 7, lines 1-3 of the specification and originally-field claim 16.

Support for the recitations of dependent claim 90 may be found, for example at page 7, line 5 and originally-filed claim 17.

The method for of independent claim 91 is described, for example, at page 1, lines 21-30 and page 8, lines 18-24 of the specification as well as the originally-filed claims 1, 3 and 4.

The method additional recitations of dependent claim 92 finds support, for example, in originally-filed claims 2 and 3.

The additional recitations of the method of dependent claim 93 finds support, for example, in originally-filed claim 5.

The recitations of dependent claim 94 finds support, for example, in the originally-filed claim 5.

The recitations of dependent claim 95 finds support, for example, at page 6, lines 8-13 and originally-filed claim 7.

The recitations of dependent claim 96 finds support , for example, at page 5, lines 6-9 in originally-filed claims 5 and 8.

The recitations of dependent claim 97 finds support, for example, at page 5, lines 11-19 in originally-filed claim 9.

The recombinant cell of independent claim 98 is described, for example, at page 6, lines 8-13; and page 7, lines 28 to page 8, line 24, as well as originally-filed claim 10.

Support for the recitations of dependent claim 99 may be found, for example, in originally-filed claim 11.

Support for the recitations of dependent claim 100 may be found, for example, in originally-filed claim 12.

The recitations of dependent claim 101 may be found, for example, in originally-filed claim 13.

The details of dependent claim 102 may be found, for example, in originally-filed claim 14.

The method of dependent claim 103 is supported, for example, by page 6, lines 24-30 of the specification as well as the originally-filed claim 15.

Support for the recitations of dependent claim 104 may be found, for example, at page 7, lines 1-3 of the specification and originally-filed claim 16.

The recitations of dependent claim 105 are described, for example, at page 7, line 5 of the specification as well as the originally-filed claim 17.

The method of independent claim 106 is described, for example, in originally-filed claims 5 and 7.

(6) Issues

The following three issues are presented for Appeal:

A. Whether the invention of claims 67-106 is supported by an adequate written description, as required by 35 U.S.C. § 112, first paragraph.

B. Whether the invention of claims 67-106 are supported by an enabling disclosure, as required by 35 U.S.C. § 112, first paragraph, such that one of ordinary skill in the art would be able to make and use the invention of the claims.

C. Whether the invention of claims 67-78 and 80-106 would have been obvious, under 35 U.S.C. § 103, to one or ordinary skill in the art from the combined teachings of EP 373962 (Backman), Belinga (Journal of Chromatography A.695 (1995) 33-40), Gilles (PNAS, 83: 5798-5802 (1986)), and Kajiyama (Biochemistry 32: 13795-13799 (1993)).

(7) Grouping of claims

The claims do not stand or fall together. Pursuant to 37 CFR § 1.192(c)(7), the appellants will provide in the argument required by 37 CFR § 1.192(c)(8) an explanation of why the claims of the groups are believed to be separately patentable for each ground of rejection.

(8) Argument

A. The invention of claims 67-106 are adequately described in the specification, as required by 35 U.S.C. § 112, first paragraph, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is nearly connected, to make and use the same. The Section 112, first paragraph, rejection of claims 67-106 stated in ¶ 4 on pages 2-4 of the Office Action dated May 2, 2003 (Paper No. 30) should be reversed. Consideration of the following in this regard is requested.

Initially, the following clarification of the Examiner's Statement of the claimed invention is provided for completeness. Specifically, the Examiner characterizes claims 67-72 as being drawn to methods for producing a polypeptide product by identifying a mutant form of a polypeptide with increased tolerance to pH or temperature, identifying a mutant form of an undesired protein with decreased tolerance to pH or temperature, transforming a host cell to express the mutant proteins, culturing said host cell, and recovering the genus of protein products that remain unaffected at a pH or temperature at which the genus of undesired proteins are denatured. See, page 2 of Paper No. 30. Moreover, the Examiner characterizes claims 73-82 and 91-97 as being drawn to methods for producing a polypeptide product by culturing a host cell that has been transformed to express the genus of polypeptide products that remain unaffected at the pH or temperature which an undesired protein is denatured and further transformed to express a genus of undesired polypeptides in mutant form that are denatured under conditions in which it desired polypeptide remains unaffected and optionally wherein the desired polypeptide is luciferase in the desired protein is adenylate kinase. See, the passage spanning pages 2-3 of Paper No. 30.

In fact, the "genus" of undesired proteins of claims 67-72, 73-82 and 91-94 are proteins which hinder the use of the polypeptide product and have activity essential for survival of the host cell or for a viable production process using a host cell. The protein of claims 73-82 are further described as cellular proteins. The appellants further note that only claims 67, 69, 70, 72 and 75 refer to pH or temperature, claims 68, 71, 76-78, 81-82 and 91-97 specifically referred temperature while claim 79 specifically refers to pH. The Board is further urged to appreciate that claim 73, 74 and 80 are not limited to

pH or temperature constraints but rather relate to culture conditions which denature the undesired product while the desired product is unaffected.

In a similar generalization of the claimed invention, the Examiner asserts that claims 83-87 and 98-102 are drawn to a recombinant cell expressing a genus of nucleic acids encoding a desired polypeptide that remains unaffected at a temperature at which an undesired protein is denatured and expressing a genus of nucleic acids encoding an undesired polypeptide in a mutant form that is denatured under conditions in which a desired polypeptide remains unaffected, and optionally wherein the desired polypeptide is luciferase in the undesired protein is adenylate kinase. See, page 5 of Paper No. 30. In fact, claims 83-87 do not refer to temperature but rather conditions in which the undesired protein is denatured and the desired polypeptide is unaffected. Claims 84 and 99 (which is dependent on claim 98) refer to luciferase and adenylate kinase whereas claims 83, 85-87, 98 and 100-102 require the "genus" of nucleic acids encoding the undesired polypeptide to encode a polypeptide which has an activity essential for survival of a host cell or for a viable production process using the host cell.

The Board is urged to appreciate that the presently claimed invention claims priority to GB 9722481.0 filed October 25, 1997. The state of the art of recombinant techniques was, as further detailed below, quite advanced at the time the present invention was made.

The Board is further urged to appreciate that a specification need not teach or disclose in detail that which is well-known in the art. *In re Myers* (CCPA 1969) 161 USPQ 668. Moreover, it is not always necessary to enumerate a plurality of species of

a claimed genus if the genus is otherwise sufficiently identified by appropriate language. *In re Grimme et al.(CCPA 1960) 124 USPQ 499* (copies attached in Appendix B).

While not believed to be necessary, the appellants' submit that one of ordinary skill in the art will appreciate that the presently claimed invention will be applicable to a wide range of protein products and nucleic acids encoding undesired polypeptides.

For instance, for any protein/polypeptide produced, one of ordinary skill will appreciate that removal of proteases, especially for long-term storage would be beneficial. This could be readily achieved using the method of the claimed invention by engineering themolabile proteases, and thermostable proteins. This removes the need for adding protease inhibitors, which tend to be quite toxic, to protein products.

Other examples include DNA processing enzymes (e.g., ligases, polymerases, gyrases, restriction enzymes) will be deleteriously affected by DNAse activity. Thus, one of ordinary skill in the art will appreciate that it would be desirable to eliminate DNAse from any such products. However, cells require DNAse in order to function and therefore this cannot be done at the recombinant production stage. At present, these enzymes are removed from inert polypeptides (such as BSA) by acetylation or methylation. However a thermally denaturable DNAse would provide a means for producing these enzymes more easily, by eliminating the further processing steps.

Elimination of specific contaminants is particularly important in the production of enzyme assay kits, where a contaminating activity could provide alternative routes for substrate degradation or that could alter the measured product. Regulatory approval for such kits may be dependent upon ensuring that an interfering polypeptide contaminant (enzyme activity) has been removed from every batch.

Other possible examples or species of the genus of undesired polypeptides referred to in the presently claimed invention include phosphates, which interfere with kinase activity measurement and therefore must be eliminated from kinases intended for this purpose. Many systems including the luciferase system rely on ATP-based measurements as a signaling system. Thus the presence of any ATPases in the reagents used in the assay will interfere with ATP-based measurements, and so complete elimination of these would be desirable. However, a cell requires ATPase to function, and therefore the method of the claimed invention would be applicable.

Finally, the Board is urged to appreciate that the presently claimed invention requires a combination of elements which where well-known and/or identified or obtainable by mere routine experimentation at the time the presently claimed invention was made. That is, the appellants have discovered a means to recombinantly produce luciferase, or other desired products, in the absence of the essential enzyme adenylate kinase, or other essential cellular components.

In a larger sense however the appellants have discovered that desired proteins may be recombinantly produced by the claimed method and using the claimed cells, in the substantial absence proteins which are otherwise undesired but have an activity that is essential for survival of the host cell or for a viable production process using the host cell. The later aspect of the presently claimed invention is important as alternative means are available to recombinantly produce polypeptides in the absence of undesired proteins which do not hinder the use of the polypeptide product or have an activity that is not essential for survival of a host cell or for viable production process using the host cell. See, page 2, lines 1-7 of the present application.

The present specification describes, as admitted by the Examiner on page 3 of Paper No. 30, examples of thermostable polypeptide products (i.e., luciferase proteins). Moreover, the Examiner admits on page 9 of Paper No. 30 that Kajiyama (Biochemistry 32: 13795-13799, of record) teaches a vector encoding a mutant thermostable luciferase wherein the mutation resulting in the thermostable luciferase is a substitution of threonine with isoleucine at position 217. The appellants further note that WO 95/25798 (Lowe et al.), cited by the Examiner, further teaches that the thermal and pH stability and the specific activity of the enzyme described by Kajiyama, and referred to by the Examiner, were increased. See, the paragraph spanning pages 1 and 2 of Lowe et al. Lowe et al. further teach that the luciferases having increased heat stability over wild-type luciferases were produced from luciferases of each of *Photinus pyralis*, *Luciola mingrelica*, *Luciola lateralis* and *Luciola cruciata*. See, page 2 of Lowe et al. Lowe further teaches alternative forms of the mutated luciferases may include changes to the amino acid sequence corresponding to amino acid 217 of the *Luciola* firefly luciferase or 215 of *Photinus pyralis* changed to a hydrophobic amino acid, such as isoleucine, leucine or valine, as described in EP 0524448. See, page 3, second full paragraph of Lowe et al. Lowe et al. further describes luciferases wherein the mutations at positions 217 or 215 are included in combination with the mutation at position 354. Lowe et al. describes methods for isolating and identifying further luciferases which are thermostable and/or pH insensitive and for methods of using the same, such as in recombinant methods. Lowe et al. further provides sequence information from which one of ordinary skill in the art may make and use the presently claimed invention without further experimentation.

The description of Lowe et al. is an example of the advanced level of ordinary skill in the art at the time the presently claimed invention was made. The appellants should not be required to describe in the present application that which was well known to one of ordinary skill in the art from the teachings of, for example, Lowe et al.

Further luciferase mutants are described in WO 96/22376 (Squirrell et al., of record). Specifically, the noted amino acids 217, 215, 354 and 356 of Lowe are described in Squirrell et al. Further mutations containing double and triple amino acid changes are also described. See, page 5, first paragraph of Squirrell et al. As with Lowe, Squirrell et al. is representative of the level of ordinary skill in the art at the time the present invention was made and the appellants should not be required to describe in the present application that which was well-known in the art.

Belinga et al. (of record) describes standard techniques for isolating and purifying luciferases and the appellants respectfully submit that identification and use of a variety of specific species having the desired characteristics of the present claims would have been routine to one of ordinary skill in the art once given the present specification. Beyond the identification of natural sources, the appellants note that Kajiyama (U.S. Patent No. 5,229,285 (a copy of which was previously provided and referred to at page 7 of the Amendment filed January 2, 2003, copy attached as Appendix C)) teaches that mutagenesis of genes encoding wild-type firefly luciferases can be affected according to methods known in the art. See, column 2, lines 52-54 of Kajiyama. Further details of recombinant technology are also described throughout Kajiyama.

With regard to the description of proteins which hinder the use of the desired polypeptide product and have activity essential for survival of the host cell or for a viable

production process using the host cell, such as adenylate kinase, the Examiner has admitted that Gilles et al. (PNAS, volume 83, pages 5798-5802, August 1986) teaches thermosensitive mutants of E.coli with a mutation in the endogenous *adk* gene encoding adenylate kinase. See, page 9 of Paper No. 30. The appellants believe further such polypeptides could be made, without undue experimentation, by recombinant means described, for example, in Kajiyama (U.S. Patent No. 5,229,285), and generally known in the art.

Liang et al. (Gene, 80, (1989) 21-28)), copy attached as Appendix D) teaches the efficient cloning of a mutant adenylate-kinase-encoding gene from *E.coli.* Liang teaches that cloning of the wild-type *adk* gene was reported as early as 1985 and that multiple mutants had apparently been obtained which contained thermolabile adenylate kinase as early as 1986. See, page 22, left column of Liang.

The appellants respectfully submit therefore that methods and materials which may be required to produce a protein which hinders the use of polypeptide product recited in the present claims which also has an activity essential for survival of a host cell or for a viable production process using a host cell, such as adenylate kinase, as presently claimed, were well-described in the art and available to one of ordinary skill in the art at the time the present invention was made. A detailed description in the present application of further species falling within the claims should not be required.

The Section 112, first paragraph, rejection of claims 67-106 stated in paragraph 4 on page 2 of Paper No. 30 should be reversed.

The following groups of claims, with regard to the Section 112, first paragraph rejection stated in paragraph 4 on page 2 of Paper No. 30, are submitted to be

separately patentable: claims 67-71; claims 72, 80, 84, 95 and 98-102; claims 81 and 96; claims 82 and 97; claims 73-79, 83, 85-94 and 103-105; and claim 106 are submitted to be each separately patentable one to the other.

Specifically, claims 67-71 require identification of mutant forms of the polypeptide product and undesired protein of the presently claimed invention such that means are known in the art for identification of proteins and the use of the same according to the presently claimed methods such that a more detailed description in the specification should not be required.

With regard to the group of claims 72, 80, 84, 95 and 90-102, the specific recitations of luciferase and adenylate kinase are clearly described in the present specification as well as well-known to those of ordinary skill in the art such that further description of embodiments in the specification should not be required to support the claims. The Section 112, first paragraph "written description" rejection is further believed to be specifically inappropriate with regard to this group of claims.

With regard to the group of claims 81 and 96, which specifically recite adenylate kinase which is thermolabile at a temperature of 37°C, the appellants note that the same is described in the present specification, the Examiner has admitted that the same as described in Gilles et al. and one or ordinary skill in the art will appreciate that adenylate kinase is a ubiquitous protein which has an activity essential for survival of a host cell or for a viable production process using a host cell, and that adenylate kinase exemplifies polypeptides which hinder the use of polypeptide products according to the presently claimed invention. This group of claims (claims 81 and 96) is submitted therefore to be separately patentable over the other identified groups of claims with

regard to the Section 112, first paragraph, rejection stated in ¶ 4, page 2 of Paper No. 30.

With regard to the group of claims 82 and 97, the Examiner has acknowledged that the present specification describes *E.coli* adenylate kinase with mutations at positions 87 or 107, as recited in the claims of this group. See, page 3, last full paragraph, of Paper No. 30. Moreover, one or ordinary skill in the art will appreciate from the specification as well as the prior art that adenylate kinase is a ubiquitous protein whose activity is essential for survival of the host cell or for a viable production process using a host cell and that adenylate kinase will likely hinder the use of a polypeptide product according to the presently claimed invention. Accordingly, the group of claims 82 and 97 are submitted to be separately patentable with regard to the Section 112, first paragraph, rejection stated in paragraph 4 on page 2 of Paper No. 30, as compared to the other identified groups of claims.

Finally, with regard to claim 106, the claim is believed to be separately patentable from the other identified groups of claims with regard to the Section 112, first paragraph, rejection stated in paragraph 4 on page 2 of Paper No. 30 as the claim combines the above-noted details of luciferase being the desired product, adenylate kinase being the undesired product which is denatured at temperatures of 37°C. As noted in detail above, each of these aspects of the invention of claim 106 is described in the present specification as well as well-known to those of ordinary skill in the art at the time the present invention was made. Accordingly, the invention of claim 106 is separately patentable from the other identified groups of claims with regard to the Section 112, first paragraph, rejection.

Reversal of the Section 112, first paragraph, rejection of claims 67-106 stated in ¶ 4 on page 2 of Paper No.3 is requested.

B. The invention of claims 67-106 are described in the present specification in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it most nearly connected, to make and use the same, as required by 35 U.S.C. § 112, first paragraph. The Section 112, first paragraph, rejection of claims 67-106 stated in ¶ 5 on page 4 of Paper No. 30 should be reversed. Consideration of the following in this regard is requested.

The Examiner's characterization of claims 67-106 on lines 6-12 of page 6 of Paper No. 30 is not understood as these claims are not so limited. Clarification is requested for the convenience of the Board.

The appellants note that the Examiner indicated in the Office Action of July 2, 2002 (Paper No. 19) that

> "The specification is enabling for a recombinant cell comprising a polynucleotide encoding a Luc that is thermostable at temperatures of 37°C or more and a polynucleotide encoding a mutant AK polypeptide that is inactivated at temperatures of 37°C or more, wherein the Luc maintains at least partial enzymatic activity and methods of making said recombinant cell or using said recombinant cell for the production of Luc." See, page 5 of Paper No. 19.

Accordingly, the appellants believe that, at a minimum, the Examiner has previously recognized that claims 81, 82, 96, 97 and 106 are supported by an enabling disclosure.

The Examiner attempts to provide an analysis on pages 5-8 of Paper No. 30 following the factors discussed in *In re Wands,* 8 USPQ2d 1400 (Fed. Cir. 1988) (copy attached as Appendix E).

As noted by the Court in *Wands*,

> "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. "The key word is 'undue,' not 'experimentation.'" The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." See, 8 USPQ2d 1404 (Citations omitted).

The appellants respectfully submit that specification, read in conjunction with the generally advanced level of ordinary skill in the art at the time the invention was made, teaches one of ordinary skill in the art how to make and use the methods and products of, at least, claims 72, 80, 81, 82, 95-102 and 106. That is, adenylate kinase and luciferase sequences were known at the time that the present invention was made; methods of making and/or screening and testing for temperature sensitive forms of adenylate kinase and luciferase were known at the time the present invention was made; and recombinant technology was available to make and use cells as presently claimed.

While some experimentation may have been required to make and use these aspects of the claimed invention, such experimentation would not have been an undue

amount. To find that the specification is enabling for only a method of producing *Photimus pyralis* luciferase with a mutation that position 354 or *Luciola* luciferase with a mutation at position 356 that is substantially free of *E. coli* adenylate kinase with a mutation at position 87 or 107, as suggested by the Examiner at page 4 of Paper No. 30, would be "strained and unduly harsh" (see, *In re Wands*, 8 USPQ2d 1400, 1406, wherein the *Wands* court similarly characterized the Board's decision to uphold the enablement rejection in *Wands*).

As noted previously, Kajiyama (U.S. Patent No. 5,229,285), and the related Biochemistry paper cited by the Examiner, describe thermostable luciferases and methods for making and screening for the same. The above-noted publications of Lowe et al. and Squirrell et al. similarly contain alternative luciferases and Belinga et al., cited by the Examiner, describes the routine purification and testing of luciferase from natural sources. These references further describe a range of temperatures at which the thermostable luciferase will be active.

With regard to the adenylate kinase aspect of the presently claimed invention, the sequence of adenylate kinase was known at the time the present invention was made, as admitted by the Examiner in citing Gilles et al. (PNAS, 83, 5798-5802, 1986). Liang et al. further describes the well-known existence of temperature sensitive mutants of adenylate kinase and demonstrates the ability of one of ordinary skill in the art to use less than undue experimentation to screen for and/or purify the same.

At a minimum therefore, the appellants submit that the Section 112, first paragraph, rejection of claims 72, 80, 81, 82, 95-102 and 106 should be reversed.

With regard to the remaining claims, as well as these above noted claims, the appellants submit that the Examiner's obviousness rejection of claims 67-78 and 80-106, further discussed below, is inconsistent with the Examiner's assertions that one of ordinary skill in the art would not have been able to make and use the presently claimed invention from the specification and generally advanced level of skill in the art. If, as the Examiner alleges,

> one would have [had] a reasonable expectation of success for transforming the *E.coli* expressing an endogenous thermolabile adenylate kinase of Gilles or *E. coli* with a disruptive *adk* gene transformed with a plasmid expressing the mutant adenylate kinsase of Gilles et al. with the vector encoding the mutant thermostable luciferase of Kajiama et al., expressing the mutant thermostable luciferase in heating the resulting cell or cell extract at a temperature of 40°C to denature and inactivate the thermolabile adenylate kinase because of the results of EP 373962, Kajiyama et al., and Gilles et al." (see, page 10 of Paper No. 30),

then the present specification, taken with the generally advanced level of skill in the art, as reflected by the Examiner's citations, must be sufficient to teach one of ordinary skill in the art how to make and use the presently claimed invention. The Section 112, first paragraph, rejection of claims 67-106 should be reversed.

The Examiner's assertions as to unpredictability in the art is not supported by any technical or scientific evidence. The Examiner's assertion that one of ordinary skill in the art would have required the specification to predict which mutations of any desired protein or luciferase will result in an increased tolerance to temperature or pH and/or the ability to predict which mutations of undesired protein or adenylate kinase will result in a decreased tolerance to temperature or pH is not realistic. See, page 7 of Paper No. 30.

Rather, as described in *In re Wands*, and quoted above, the standard of reasonableness must be applied and "routine screening" is an example of a reasonable amount of experimentation. See, 8 USPQ2d 1404. Accordingly, one of ordinary skill in the art would require no more than to use routine methods of screening luciferase and adenylate kinase enzymes, for example, to make and use the presently claimed invention. In fact, random mutagenses of the genes of wild-type firefly luciferase, as described in Kajiyama (U.S. Patent No. 5,229,285) is a viable option for producing mutant luciferases useful in the presently claimed invention. Similar means may be used, for example, to produce mutated adenylate kinases useful in the presently claimed invention. One of ordinary skill in the art would not require, and the appellants should not be required to provide, "detailed knowledge of the ways in which the proteins' structure relates to its function" (see, page 7 of Paper No. 30) to satisfy the requirements of Section 112, first paragraph.

The Examiner's conclusion as to whether the combined use of mutant adenylate kinases and mutant luciferases, according to the claimed invention, would require an undue amount of experimentation is unsupported and, with due respect, submitted to be inappropriate as a basis for maintaining the Section 112, first paragraph of claim 67-106.

Similarly, the Examiner's assertion that "it is not routine in the art to screen for all mutant proteins with an increased and decreased tolerance of temperature or pH, as encompassed by the instant claims" (see, page 7 of Paper No. 30) is not believed to be relevant. One of ordinary skill in the art is not required to screen for all mutant proteins according to the claims to make and use the claimed invention but rather only a mutant

form of a desired peptide and an undesired polypeptide, once one of ordinary skill in the art identifies a peptide which is desired, and to which the production of the same is being hindered by the further undesired peptide which has an activity essential for survival of the host cell or for a viable production process using a host cell. That is, the Examiner's comments appear to ignore the fact that the presently claimed invention is not directed to the production of any peptide in conjunction with the simultaneous production of any undesired peptide but rather the claimed invention specifies that a desired polypeptide is produced in an environment where a second undesired peptide is also produced wherein the undesired peptide hinders the use of polypeptide product which is desired and the undesired peptide has an activity essential for survival of the host cell or for a viable production process using a host cell. Once these desired and undesired peptides are identified by one of ordinary skill in the art, screening methods are routine for identifying and using mutant forms of the desired and undesired peptides. The appellants should not be required to teach all such peptides, as suggested by the Examiner, as recombniant techniques are well known to those of ordinary skill in the art. Moreover, the appellants should not be limited to the specifically disclosed mutants wherein only routine experimentation may be required to make and use the claimed invention.

The claims are submitted to be supported by an enabling disclosure and reversal of the Section 112, first paragraph, rejection of claims 67-106 stated in paragraph 5 on page 4 of Paper No. 30 is requested.

The appellants note that claims 67-106 do not stand or fall together with regard to the Section 112, first paragraph, rejection stated in paragraph 5 on page 4 of Paper

No. 30. Rather, the following groups of claims are submitted to be separately patentable: claims 67-71; claims 72, 80, 84, 95 and 98-102; claims 81 and 96; claims 82 and 97; claims 73-79, 83, 85-94 and 103-105; and claim 106.

Claims 67-71 are submitted to be separately patentable with regard to the Section 112, first paragraph, rejection as one of ordinary skill in the art would have been able to identify the noted proteins, as required by the claims, without undue experimentation. The art of record, for example, describes the advance skill in this art to identify temperature and/or pH sensitive mutants, and the use of recombinant methods to produce proteins and means useful for the same. The use of the combination of proteins identified according to these claims would not have required an undue amount of experimentation.

Claims 72, 80, 84, 95 and 98-102 are submitted to be separately patentable with regard to the Section 112, first paragraph, rejection as the production of use of the claimed invention with the recited luciferase and adenylate kinase enzymes would not require an undue amount of experimentation as the art is replete with examples of the routine ability of one of ordinary skill in the art to make and use these enzymes. The combination of luciferase and adenylate kinase in the methods and products of claim 72, 80, 84, 95 and 98-102 would not require an undue amount of experimentation.

Claims 81 and 96 are submitted to be separately patentable with regard to the Section 112, first paragraph, rejection as adenylate kinase which is thermolabile at a temperature of 37°C is obtainable from the art without undue experimentation and the use of the same in a presently claimed methods would not require undue experimentation.

The invention of claim 82 and 97 are submitted to be separately patentable with regard to the Section 112, first paragraph, rejection as the Examiner has admitted the adenylate kinase recited in these claims is taught by the present specification. The inclusion of the same in the methods of claims 82 and 97, which are dependent on claims 81 and 96, respectively, and therefore also include the luciferase recited in claims 80 and 95, respectively, would not require an undue amount of experimentation. Claims 82 and 97 are believed to be separately patentable.

Finally, the method of claim 106, which includes the use of luciferase and adenylate kinase which are thermostable and denatured at temperatures of 37°, respectively, are submitted to be supported by the specification and generally advanced level of skill in the art.

Reversal of the Examiner's Section 112, first paragraph, rejection stated in paragraph 5 of Paper No. 30 is requested.

C. The invention of claims 67-78 and 80-106, would not have been obvious, under 35 U.S.C. Section 103, to one of ordinary skill in the art from the combined teachings of EP 373962 (Backman), Belinga (Journal of Chomatography A, 695:33-40 (1995)), Gilles (PNAS, 83: 5798-5802, 1986) and Kajiyama (Biochemisty 32: 13795-13799, 1993). The Section 103 rejection should be reversed. Consideration of the following comments in this regard is requested.

The Examiner has failed to establish a *prima facie* case of obviousness.

As described previously, the presently claimed invention requires a combination of the use of the desired protein which is stable under given conditions and an

undesired protein which is unstable under the same conditions wherein the undesired protein is one which hinders the use of the desired protein and has activity essential for survival of the host cell or for a viable production process using a host cell. Exemplified desired proteins include luciferase and undesired proteins are exemplified in the present application by adenylate kinase. The Examiner has combined the cited four references through an inappropriate use of hindsight.

The Examiner asserts that one of ordinary skill in the art would have been motivated to combine the components of Gilles' adenylate kinase and Kajiyama's luciferase because Belinga teaches a desire to remove contaminating adenylate kinase from luciferase preparations for bioluminescence. The Examiner further asserts that one of ordinary skill in the art would have reasonably expected to be successful due to "the results of EP 373962, Kajiyama et al., and Gilles et al." See, page 10 of Paper No. 30.

The results of Backman (EP 0373962) however relate to the use of thermostable enzymes wherein a thermostable form of an enzyme is used in a recombinant production method followed by application of extreme heat to denature all but the desired peptide. See, column 2, line 53 through column 3, line 17 of Backman.

Backman does not teach luciferase production. None of the cited art teaches a luciferase which could be used in Backman's process at the temperatures described therein (i.e., 80-95°C, see, column 3, lines 14-15 of Backman).

More importantly, the process of Backman does not require production or engineering of proteins or polypeptides as required by the presently claimed invention. That is, a luciferase produced according to the method of Backman would only require

production of thermostable form of luciferase in a mesophilic host cell, culturing the mesophilic host cell to produce the thermostable luciferase and purify the thermostable luciferase by at least heating to a temperature sufficient to inactivate the unwanted contaminants but not sufficient to inactivate the thermostable luciferase. See, column 2, lines 21-37 of Backman.

Backman does not describe or suggest a simultaneous production of a desired polypeptide in a mutant form which has increased tolerance to a particular reaction condition, such as pH or temperature, as well as a mutant form or an undesired protein which has decreased tolerance to the reaction condition as compared to a wild-type form of the undesired protein and wherein the undesired protein hinders the use of the polypeptide product and has an activity that is essential for survival of a host cell or for a viable production process using a host cell. Backman therefore provides a process wherein the identification or use of the mutant of the undesired peptide of the presently claimed invention would not be required. Combination of Gilles and/or Belinga, which describe the identification of adenylate kinase, with Backman therefore would not be logical to one of ordinary skill in the art wishing to produce luciferase in a process of Backman.

Kajiyama teaches a mutant thermostable luciferase and also that "one of the most important goals of protein engineering is to produce mutant enzymes which have greater thermostability than the parent proteins". See, page 13795, left column, second full paragraph of Kajiyama. One of ordinary skill in the art reading Kajiyama and Backman would, at best, be motivated to produce luciferases which could be sufficiently thermostable to be expressed in the mesophilic host cells of Backman. The preferred

temperatures of purification according to Backman is 80°C - 95°C. See, column 3, lines 14 and 15 of Backman. The thermostable mutant luciferase of Kajiyama was tested at 50°C and found to have a half life which was roughly 8-10 times longer than that of the wild-type luciferase. See, page 13796, right column, first full paragraph, and Figure 3 of Kajiyama. Even if one of ordinary skill in the art reading Kajiyama and Backman would have been motivated, at least, to produce further luciferase mutants with even greater thermostability, such is not the subject of the presently claimed invention.

The Board will appreciate that luciferases are notoriously thermolabile enzymes. The sort of thermostability that a company like Promega, for example, can obtain is illustrated in WO 99/14336, a copy of the front page of which is attached as Appendix F. Even following a program of directed evolution, resulting in multiple mutations, the thermostability of the enzyme is still far short of that which would be required to be produced in a Backman type process. The Board is specifically requested to note that the test temperature quoted in the abstract is still only 50°C, and even at this temperature, a half life of at least 2 hours is regarded as being a great increase. A skilled person would not therefore, realistically consider trying to produce further luciferase mutants for use in a Backman process.

The Board is urged to appreciate that the enzymes of Backman are derived from bacteria isolated from hot springs. This bacteria is able to survive in the sorts of temperatures in which most life cannot survive. Backman is relying on the extreme thermostability which enzymes from these organisms have to operate his method. Luciferase on the other hand is derived from conventional species which survive at ambient temperatures, such as fireflies and gloworms. Achieving stability levels

equivalent to those of an enzyme required by Backman would be quite an unreasonable expectation.

Gilles teaches thermosensitive mutants of *E. coli* with a mutation in the endogenous *adk* gene encoding adenylate kinase. The thermosensitive adenylate kinase of Gilles is irreversibly inactivated by incubation of crude extracts at 40°C. See, page 5798, left column, last paragraph of Gilles. One of ordinary skill in the art reading Gilles and Backman, with or without Kajiyama, would have presumably been interested in producing adenylate kinase in a Backman type process and would have been frustrated by the decrease in thermostability of adenylate kinase taught by Gilles, as Backman is interested in and requires thermostable enzymes. One of ordinary skill in the art therefore and would not have been taught by Gilles how to make thermostable enzymes for expression in the mesophilic host cells of Backman. More importantly, the appellants require thermolabile adenylate kinase whereas Backman suggests, at best, production of thermostable enzymes in mesophilic hosts at, preferably, 85-90°C. Accordingly, it is unclear to the appellants how or why one of ordinary skill in the art would combine Gilles and Backman, with or without Kajiyama and Belinga, to make the presently claimed invention.

Finally, the Examiner combines Belinga and asserts that the teaching and the necessity of removing adenylate kinase from luciferase in Belinga would have motivated one of ordinary skill in the art to combine the four cited references to allegedly produce the presently claimed invention.

The appellants respectfully submit however that the Examiner's interpretation of the cited art is contrary to at least Backman which describes the expression of a single

gene encoding a heterologous thermostable enzyme. One of ordinary skill in the art interested in producing luciferase in the absence of adenylate kinase, as suggested by the Examiner, would have taken from the Examiner's combination of references that a much more thermostable luciferase, as compared to Kajiyama, is required for production in the method of Backman. Heating and the purification step of Backman would eliminate the contaminating adenylate kinase which the Examiner asserts is taught by Belinga but would also eliminae any luciferase product. The appellants note that Belinga reports "a rapid and convenient procedure" for purifying luciferase from adenylate kinase. The procedure of Belinga "consists of precipitation of firefly luciferase with PEG 20,000, followed by affinity chromatography on an Orange A gel, which binds many interfering enzymes of the bioluminescent reaction not luciferase, which can therefore obtained without a specific allusion step." See, the paragraph spanning pages 33 and 34 of Belinga. The method of Belinga does not require the use of luciferase mutants or adenylate kinase mutants or recombinant methods. Moreover, Belinga reports that a luciferase preparation with 95% of total activity and free from interfering enzymes such as adenylate kinase and nucleocide diphosphate kinase" was produced. See, page 38, right column, second full paragraph of Belinga. The appellants respectfully submit therefore that one of ordinary skill in the art would not have been motivated to provide further methods for purifying luciferase from adenylate kinase in view of Belinga's complete purification.

The Section 103 rejection of claims 67-78 and 80-106 should be reversed.

The appellants note that claims 67-78 and 80-106 do not stand or fall together with regard to the Section 103 rejection. Rather, the following groups of claims are

submitted to be separately patentable: claims 67-71; claims 72, 80, 84, 95 and 98-102; claims 81 and 96; claims 82 and 97; claims 73-78, 83, 85-94 and 103-105; and claim 106.

Claims 67-71 are submitted to be separately patentable with regard to the Section 103 rejection as the cited art fails to teach or suggest the combined identification of the noted proteins, as required by the claims. Rather, the art of record, such as Kajiyama, describes the identification of a thermostable luciferase, as opposed to the combination of two enzymes, or proteins in general, as required by these claims. Moreover, Backman teaches the use of a thermostable protein in a mesophilic organism to purify the protein from other cellular components by applying extreme heat. The use of the combination of proteins identified according to these claims would not have been obvious from the Examiner's combination of cited art.

Claims 72, 80, 84, 95 and 98-102 are submitted to be separately patentable with regard to the Section 103 rejection as the production of use of the claimed invention with the recited luciferase and adenylate kinase enzymes, in combination, would not have been obvious from the cited art. As noted above, Belinga teaches a method of complete purification of luciferase such that the recombinant method of the presently claimed invention is yet a further, patentably distinct, method of producing luciferase free of adenylate kinase. The post production purification process of Belinga and/or the production of a single thermostable protein according to Backman would not have made the presently claims process and products, which require production of mutated forms of luciferase and adenylate kinase, obvious. The combination of luciferase and

adenylate kinase in the methods and products of claim 72, 80, 84, 95 and 98-102 would not have been obvious in view of the cited art.

Claims 81 and 96 are submitted to be separately patentable with regard to the Section 103 rejection as the use of adenylate kinase which is thermolabile at a temperature of 37°C is yet a further refinement of the presently disclosed invention which is not suggested by the cited art. Specifically, Backman teaches the production of proteins which are stable, preferably, at a temperature of about 85°C, which teaches away from the use or production of a thermolabile adenylate kinase of this group of claims. Claims 81 and 96 are submitted to be separately patentable over the cited art.

The invention of claim 82 and 97 are submitted to be separately patentable with regard to the Section 103 rejection as the mutant of these claims are submitted to be separately patentable species within the claims from which they depend. The inclusion of the same in the methods of claims 82 and 97, which are dependent on claims 81 and 96, respectively, and therefore also include the luciferase recited in claims 80 and 95, respectively, would not have been obvious from the cited art. Claims 82 and 97 are believed to be separately patentable.

Finally, the method of claim 106, which includes the use of luciferase and adenylate kinase which are thermostable and denatured at temperatures of 37°, respectively, are submitted to be separately patentable as preferred combinations of the elements which exemplify the presently disclosed invention.

The Section 103 rejection of claims 67-78 and 80-106 should be reversed.

In conclusion, the application is submitted to be in condition for allowance. Reversal of the Final Rejection and passage of the subject application to issue are earnestly solicited.

Respectfully submitted,

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APPENDIX A

Claims on Appeal

APPENDIX A

Claims on Appeal

APPENDIX A

Pending Claims 67-106 in Serial No. 09/529,722

67. A method for producing a polypeptide product which is substantially free of a specific undesired protein that hinders the use of the polypeptide product, wherein the undesired protein has activity that is essential for survival of a host cell or for a viable production process using a host cell, the method comprising

(a) identifying a mutant form of said polypeptide product which has increased tolerance to a particular reaction condition selected from pH or temperature, than a corresponding wild-type polypeptide product,

(b) identifying a mutant form of said undesired protein which has decreased tolerance to said reaction condition than a wild-type form of said undesired protein, and is denatured under conditions at which the mutant form of the polypeptide product identified in step (a) is stable,

(c) transforming a host cell so that it expresses the mutant form of the polypeptide product identified in step (a),

(d) further transforming the host cell so that it expresses the undesired protein only in the mutant form identified in step (b),

(e) culturing said host cell and recovering the desired product, wherein either the host cell culture or the recovered product is subjected for a sufficient period of time to conditions at which the undesired protein is denatured but the polypeptide product remains unaffected.

68. A method for producing a polypeptide product which is substantially free of a specific undesired protein that hinders the use of the polypeptide product, wherein the

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undesired protein has activity that is essential for survival of a host cell or for a viable production process using a host cell, the method comprising

(a) identifying a mutant form of said polypeptide product which has increased thermostability than a corresponding wild-type polypeptide product,

(b) identifying a mutant form of said undesired protein which has decreased thermostability than a wild-type form of said undesired protein, and is denatured under conditions at which the mutant form of the polypeptide product identified in step (a) is stable,

(c) transforming a host cell so that it expresses the mutant form of the polypeptide product identified in step (a),

(d) further transforming the host cell so that it expresses the undesired protein only in the mutant form identified in step (b),

(e) culturing said host cell and recovering the desired product, wherein either the host cell culture or the recovered product is subjected for a sufficient period of time to a temperature at which the undesired protein is denatured but the polypeptide product remains unaffected.

69. A method according to claim 67 or claim 68 wherein the step (a) is carried out by mutating colonies of host cells using non-specific methods, differentially screening colonies that are able to grow at 25°C but not able to grow at 37°C, and screening these colonies for activity of the specific undesired protein at various temperatures.

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70. A method according to claim 67 or claim 68 wherein in step (d) a host cell is transformed so that chromosomal genes expressing the said undesired protein are inactivated, and a gene which expresses the mutant form of the protein identified in step (b) is introduced into the host cell on a plasmid.

71. A method according to claim 68 wherein the temperature used in step (e) is 37°C.

72. A method according to claim 67 or claim 68 wherein the polypeptide product is a luciferase, and the specific undesired protein is adenylate kinase.

73. A method for producing a polypeptide product which is substantially free of a specific undesired cellular protein that hinders the use of the polypeptide product, wherein the undesired protein has activity that is essential for survival of a host cell or for a viable production process using the host cell, the method comprising culturing a host cell which has been transformed so that it expresses said polypeptide product and further transformed so that it expresses said undesired protein only in a mutant form which form has the said activity of the corresponding native protein under culture conditions but is denatured under conditions at which the said polypeptide product remains unaffected; and recovering the desired product, wherein either the host cell culture or the recovered product is subjected for a sufficient period of time to conditions

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under which the undesired protein is denatured but the polypeptide product remains unaffected.

74. A method according to claim 73 wherein the host cells are cultured for a period which is sufficient to allow production of polypeptide product, and then a batch of said culture is subjected to the said conditions under which the undesired protein is denatured, and the polypeptide product is recovered from the said batch.

75. A method according to claim 73 wherein the conditions at which the undesired protein is denatured and the polypeptide product remains unaffected are a predetermined temperature or pH conditions.

76. A method according to claim 75 wherein the conditions at which the undesired protein is denatured and the polypeptide product remains unaffected are a predetermined temperature.

77. A method according to claim 76 wherein the predetermined temperature is 37°C.

78. A method according to claim 77 wherein the host cell or the recovered product is subjected to a temperature of from 37°C, up to the temperature at which the desired polypeptide product is denatured.

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79. A method according to claim 73 wherein the conditions at which the undesired protein is denatured and the polypeptide product remains unaffected are pH conditions.

80. A method according to claim 73 wherein the desired polypeptide product is luciferase and the undesired protein is adenylate kinase.

81. A method according to claim 80 wherein the adenylate kinase is thermolabile at a temperature of 37°C.

82. A method according to claim 81 wherein the adenylate kinase includes mutations at amino acids 87 or 107 in the sequence of E. coli adenylate kinase.

83. A recombinant cell which has been transformed so that it expresses a first nucleotide sequence which encodes a desired polypeptide under the control of regulatory elements which allow expression of said polypeptide, and is further transformed so that it expresses a specific protein which is undesirable as a contaminant in preparations of said polypeptide product but wherein the undesired protein has activity that is essential for survival of a host cell or for a viable production process using the host cell, only in mutated form such that the protein expressed is denatured under conditions in which the polypeptide product remains unaffected.

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84. A recombinant cell according to claim 83 wherein the said desired polypeptide comprises a luciferase and the said undesired protein comprises adenylate kinase.

85. A recombinant cell according to claim 83 which further comprises at least one selection marker.

86. A recombinant cell according to claim 83, which comprises a prokaryotic cell.

87. A recombinant cell according to claim 83 which comprises a recombinant E. coli cell.

88. A method for producing a recombinant cell according to claim 83 which method comprises in any order (a) transforming a host cell with a vector which encodes said undesired protein in a form which is denatured under given conditions, subjecting transformants to said conditions and detecting those in which protein product is denatured, and (b) transforming said host cell with a vector which encodes a desired polypeptide which is unaffected under said conditions and a first selection marker, and using the first selection marker to detect stable transformants.

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89. A method according to claim 88 wherein the vector which encodes said undesired protein in a form which is denatured under given conditions further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

90. A method according to claim 89 wherein said selection markers comprise particular different antibiotic resistance genes.

91. A method for producing a polypeptide product which is substantially free of a specific undesired protein that hinders the activity of the polypeptide product, wherein the undesired protein has activity that is essential for survival of a host cell or for a viable production process using the host cell, the method comprising culturing a host cell which has been transformed so that it expresses said polypeptide product and further transformed so that it expresses said undesired protein only in a mutant form which form has the said activity of the corresponding native protein under culture conditions but is denatured at temperatures at which the said polypeptide product remains unaffected; and

recovering the desired product, wherein either the host cell culture or the recovered product is subjected for a sufficient period of time to a temperature at which the undesired protein is denatured but the polypeptide product remains unaffected.

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92. A method according to claim 91 wherein the host cells are cultured for a period which is sufficient to allow production of polypeptide product, and then a batch of said culture is subjected to said conditions of temperature under which the undesired protein is denatured, and the polypeptide product is recovered.

93. A method according to claim 91 wherein the temperature is 37°C.

94. A method according to claim 91 wherein the host cell or the recovered product is subjected to a temperature of from 37°C, up to the temperature at which the desired polypeptide product is denatured.

95. A method according to claim 91 wherein the desired polypeptide product is luciferase and the undesired protein is adenylate kinase.

96. A method according to claim 95 wherein the adenylate kinase is thermolabile at a temperature of 37°C.

97. A method according to claim 96 wherein the adenylate kinase includes mutations at amino acids 87 or 107 in the sequence of E. coli adenylate kinase.

98. A recombinant cell which has been transformed so that it expresses a first nucleotide sequence that encodes a desired polypeptide under the control of regulatory

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elements which allow expression of said polypeptide, and is further transformed so that it expresses a specific undesired protein that hinders the use of the polypeptide product but has activity that is essential for survival of a host cell or for a viable production process using the host cell, only in mutated form such that the protein expressed is denatured at a temperature at which the polypeptide product remains unaffected.

99. A recombinant cell according to claim 98 wherein the said desired polypeptide comprises a luciferase and the said undesired protein comprises adenylate kinase.

100. A recombinant cell according to claim 98, which further comprises at least one selection marker.

101. A recombinant cell according to claim 98, which comprises a prokaryotic cell.

102. A recombinant cell according to claim 98 which comprises a recombinant E. coli cell.

103. A method for producing a recombinant cell according to claim 98 which method comprises in any order (a) transforming a host cell with a vector which encodes said undesired protein in a form which is denatured under given temperature conditions,

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subjecting transformants to said temperature conditions and selecting those in which protein product is denatured, and (b) transforming said host cell with a vector which encodes a desired polypeptide which is unaffected under said temperature conditions and a first selection marker, and using the first selection marker to detect stable transformants.

104. A method according to claim 103 wherein the vector which encodes said undesired protein in a form which is denatured under given temperature conditions further comprises a second selection marker which is different to said first selection marker, and stable transformants are selected.

105. A method according to claim 104 wherein said selection markers comprise particular different antibiotic resistance genes.

106. A method for producing a luciferase which is substantially free of adenylate kinase, the method comprising culturing a host cell which has been transformed so that it expresses a luciferase which is thermostable at 37°C, and expresses adenylate kinase only in a mutant form which form is denatured at temperatures of 37°C; and recovering the luciferase, wherein either the host cell culture or the recovered luciferase is subjected for a sufficient period of time to temperatures at which the adenylate kinase is denatured but the luciferase remains unaffected.

APPENDIX B

In re Myers (CCPA 1969) 161 USPQ 668
In re Grimme et al.(CCPA 1960) 124 USPQ 499

Court of Customs and Patent Appeals

In re MYERS

No. 8035 Decided May 22, 1969

PATENTS

1. Specification—Sufficiency of disclosure (§ 62.7)

Omission from specification's disclosure is not fatal where disclosure is sufficient to enable those skilled in the art to practice the invention; specification is directed to those skilled in the art and need not teach or point out in detail that which is well known in art. —In re Myers (CCPA) 161 USPQ 668.

2. Claims—Broad or narrow—In general (§ 20.201)

"A bonded hard metal carbide rim" is not unduly broad since such a limitation defines an article which is old and well known to one skilled in the art.—In re Myers (CCPA) 161 USPQ 668.

3. Construction of specification and claims—By specification and drawings—In general (§ 22.251)

Patent claims are construed in light of specification and understanding thereof by those skilled in that art to whom they are addressed. — In re Myers (CCPA) 161 USPQ 668.

4. Claims—Broad or narrow—In general (§ 20.201)

Claims — Indefinite — Mechanical (§ 20.556)

In claiming a mechanical combination, applicant is not necessarily limited to specific composition which he discloses as the material for making up each and every element of combination; if every element in mechanical combination claim were required to be so specific as to exclude known materials known to be inoperative and which even those not skilled in the art would not try, claims would fail to comply with 35 U S.C. 112 because they would be so detailed as to obscure, rather than particularly point out and distinctly claim, the invention.—In re Myers (CCPA) 161 USPQ 668.

5. Claims — Broad or narrow — In general (§ 20.201)

Applicant described his invention as comprehending use therein of any metal capable of performing specific function in specific combination, and disclosed specifically two metals having such capability; claims do not comprehend a class of metals of any greater breadth than is comprehended by described invention; since it is clear from description that applicant regards as his invention specific combination with epoxy resin and any metal powder capable of stated function, limitation of "a powdered metal" is not unduly broad.—In re Myers (CCPA) 161 USPQ 668.

6. Specification—Claims as disclosure (§ 62.3)

Claims contained in application as originally filed may be considered part of disclosure of application.—In re Myers (CCPA) 161 USPQ 668.

Particular patents—Slitter Knives

Myers, Slitter Knives, claims 16 to 19 of application allowed; claim 20 refused.—In re Myers (CCPA) 161 USPQ 668.

Appeal from Board of Appeals of the Patent Office.

Application for patent of George E. Myers, Serial No. 847,836, filed Oct. 21, 1959; Patent Office Group 150. From decision rejecting claims 16 to 20, applicant appeals. Affirmed as to claim 20; reversed as to claims 16 to 19; Neese, Judge, concurring with opinion; Worley, Chief Judge, with whom Almond, Judge, joins, dissenting with opinion.

JOHN M. WEBB, Pittsburgh, Pa. (SPENCER B. MICHAEL, Washington, D. C., of counsel) for appellant.

JOSEPH SCHIMMEL (S. WM. COCHRAN of counsel) for Commissioner of Patents.

Before WORLEY, Chief Judge, and RICH, SMITH, ALMOND, and BALDWIN, Associate Judges.

BALDWIN, Judge.

This appeal is from the decision of the Patent Office Board of Appeals[1] affirming the examiner's rejection of claims 16-20, the only remaining claims in appellant's application.[2] The board affirmed the rejection of certain of those claims on several grounds: (1) claims 16-20, as based on an insufficient disclosure, under 35 U.S.C. 112; (2) claims 16-20, as unduly broad and in-

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[1] Consisting of Messrs. Rosa and Behrens, Examiners-in-Chief, and Bendett, Acting Examiner-in-Chief, opinion by Mr. Behrens.

[2] Serial No. 847,836, filed October 21, 1959, for "Slitter Knives." The application, originally entitled "Slitter Knives and Their Manufacture," was amended, pursuant to a restriction requirement, to cancel the method-of-making claims and to eliminate reference to that method in the description of the claimed invention, as appellant elected to prosecute claims to the slitter knife.

definite, under 35 U.S.C. 112; (3) claims 16-20, as obvious in view of Van der Pyl,[3] Nestor,[4] Kistler et al.,[5] and Voegeli-Jaggi,[6] under 35 U.S.C. 103; and claims 17, 19 and 20, as drawn to new matter. No claims have been allowed.

The Invention

The invention relates to an improved rotary or wheel-type *slitter knife* for *cutting* metal strip and sheet and is illustrated below as having a hard metal carbide blade or cutting rim 10, 10a, preferably of tungsten carbide, separated from and mounted on a hardened steel bushing 11, 11a by means of a supporting member 12, 12a formed in situ by curing or hardening a mixture of powdered or finely divided metal, such as steel or aluminum, and epoxy or epoxy-polyamide resin having an epoxide equivalent (grams of resin containing one gram equivalent of epoxide) of 400 or less.

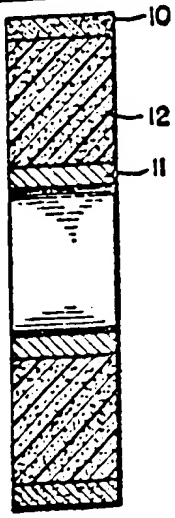

Fig. 1

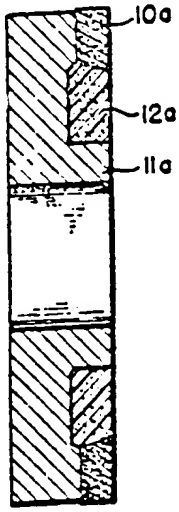

Fig. 2

In describing the preferred embodiment, the specification states:

> The supporting member must form a strong bond with both the tungsten carbide cutting rim and the steel bushing, it must possess substantial strength, particularly under compression, and it should not shrink during curing. These properties are obtained by using a mixture of powdered or finely-divided metal, such as steel or aluminum, and an epoxy or epoxy-polyamide resin. These resins have excellent dimensional stability during the curing process and provide a strong bond with the rim and the bushing. When coupled with powdered metal, they yield a supporting member with excellent compressive strength. I have found that a mixture of 80% powdered steel and 20% resin works very satisfactorily. However, these proportions may be varied depending on the strength desired.

The Claims

The appealed claims read:

16. A rotary slitter knife for slitting metal strip and sheet comprising a bonded hard metal carbide rim as the cutting element of said knife, said rim being mounted on a metal bushing by a supporting member disposed between at least a part of said bushing and said rim, said supporting member consisting essentially of a hardened material of a powdered metal and an epoxy resin, said resin being characterized by high dimensional stability during curing and good bonding properties, said supporting member effecting a bond with said rim and said bushing.

17. The knife of Claim 16 wherein said resin has an epoxide equivalent no greater than 400.

18. The knife of Claim 16 wherein said supporting member is substantially about 80% powdered metal and

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[3] U. S. Patent 2,150,886, issued March 14, 1939.

[4] U. S. Patent 2,862,806, issued December 2, 1958.

[5] U. S. Patent 2,189,734, issued February 6, 1940.

[6] U. S. Patent 2,070,734, issued February 16, 1937.

substantially about 20% resin, said powdered metal being selected from a group consisting of steel and aluminum.

19. The knife of Claim 16 wherein said bonded hard metal carbide consists essentially of tungsten carbide and said resin has an epoxide equivalent no greater than 400.

20. The knife of Claim 19 wherein the binder for said bonded hard metal carbide is a metal selected from the group consisting of cobalt and nickel.

The References

The reference patents, all relating to "abrasive" or "grinding" articles and *not* metal "slitting" or "cutting" tools, are summarized below.

Van der Pyl discloses a *grinding* wheel having an outer *grinding* rim formed of diamond grains bonded with a synthetic resin, and an inner supporting member formed of metal powder, preferably aluminum, bonded with natural or synthetic resins. Suitable natural or synthetic resins include phenolformaldehyde resin, any alkyd resin, thermoplastic resins, and shellac or the like.

Nestor discloses an *abrasive* wheel made by whirling a rotating mold containing a suspension of *abrasive grit*, preferably aluminum oxide grains, and a resin binder, preferably epoxide resin having an "epoxy number approximately 192 grams per epoxide equivalent." Centrifugal force causes the *abrasive grains* to be arranged uniformly at the periphery leaving a "clear inner resin ring."

Kistler et al. relates to a molded *abrasive* article or *grinding* wheel. The abrasive portion is disclosed as composed of *abrasive grains*, such as, "any of the hard carbides, for example boron carbide or tungsten carbide" bonded with a novel synthetic resin formed by copolymerizing a suitable ester of acrylic acid or an alpha substituted acrylic acid with a compatible polymerizable hardening agent. The resin is softer than phenol-formaldehyde and more heat resistant than shellac or rubber.

Voegeli-Jaggi discloses a *grinding* wheel comprising an outer *grinding* portion formed of an *abrasive* grit embedded in a phenolic resin, mounted on an iron support by means of a supporting portion which "is formed of artificial resin such as phenolic condensation product resin and is in the form of a hollow cylinder or disc."

The Rejections

Insufficient Disclosure

In support of his rejection of claims 16-20 as being based on an insufficient disclosure, the examiner stated in his Answer:

> * * * Since appellant's disclosure as originally filed fails to identify the bond material for the tungsten carbide rim "10", the specification * * * fails to set forth a complete written description of the best mode contemplated by the inventor of carrying out his invention.

In sustaining, the board noted appellant's "failure to describe the tungsten carbide rim material" and the absence of "a statement in the original specification to the effect that a *bonded* carbide rim was employed." [Emphasis supplied.]

Unduly Broad and Indefinite

In rejecting the claims "as not particularly pointing out and distinctly claiming the invention as required by 35 U.S.C. 112," the examiner said:

> Claim 16 is considered unduly broad and indefinite in the recited limitations of (1) "a bonded hard metal carbide rim" and (2) "a powdered metal". In the first recited limitation, the identity of the bond * * * is indefinite and would include resins and ceramics as the binder * * *. In fact, the limitation reads on a self-bonded metal carbide rim. The affidavits * * * disclose the use of an iron-group metal as essential for bonding the hard carbide particles. [The board noted that (1) applied only to claims 16-19.] The second recited limitation above, includes the powdered metals of cobalt, nickel, iron and silver in supporting member "12" which are not taught by appellant as being operative. * * * [A]luminum and steel are the only two specific metal powders identified as operative * * *. [The board noted that (2) applied only to claims 16, 17, 19 and 20 and "that the term 'powdered metal' is broad enough to refer to powdered sodium, or litnium, for instance, apparently unsuitable."]

Obviousness

The examiner rejected claims 16-20 as unpatentable over Van der Pyl in view of Nestor, Kistler et al. and Voegeli-Jaggi. The examiner's application of the references to the claims is summarized:

> Van der Pyl discloses an inner non-grinding ring consisting of aluminum powder and a synthetic resin bonded to an outer *abrading* rim of diamond grains in a synthetic resin bond. Kistler et al. discloses employing tungsten carbide or diamonds as

the *abrasive* grains in molding resin-bonded *grinding* wheels. Nestor discloses an *abrasive grinding* wheel consisting of an epoxy ring and an outer *grinding* rim consisting of *abrasive* grains and an epoxy resin. Substituting tungsten carbide particles for the diamond grains in the outer rim of Van der Pyl and employing an epoxy resin as the synthetic resin in the inner ring thereof would be obvious expedients in view of Kistler et al. and Nestor. Employing a metal bushing in Van der Pyl's *abrasive* wheel would also be obvious in view of Voegeli-Jaggi. [Emphasis added.]

In sustaining, the board opined that:

> * * * the references demonstrate that the expedient of joining a hard bonded rim to a sleeve or shaft by means of a thermosetting resin in a powdered metal composition would have been obvious. The difference in operation between a rotary slitter knife and an abrasive wheel is not a controlling consideration in this case. The record does not suggest that the problem of bonding the hardened rim to a supporting sleeve or shaft is different in these two types of wheels.

New Matter

The board sustained the rejection of certain claims as being drawn to new matter

> * * * in the limitation "no greater than 400" characterizing the epoxide equivalent in claims 17 and 19, and in the expression "a metal selected from the group consisting of cobalt and nickel" in claim 20.

In so holding, the board was of the opinion that:

> * * * the epoxide equivalent of "no greater than 400" is new to the application as a description of the final product. The specification, page 4, refers to an epoxy resin equivalent "of 400 or less" but this reference is directed to the liquid resin prior to curing, and is intended to insure liquidity at room temperature, a process and not a product feature.
>
> We find no basis in the original application for the reference to cobalt and nickel. Assuming that iron group metals have been employed as bonding materials in this art, this would not give appellant the option of selecting cobalt and nickel for monopoly after the filing date of the application.

Opinion

We believe the board's affirmance of the rejection of claims 16-20 to be in error, excepting the rejection of claim 20 as being drawn to new matter.

Insufficient Disclosure

[1] The original application contained no express statement that the carbide rim is "bonded" nor did it disclose the nature of the binding material. However, an omission is not fatal where, as here, the disclosure is sufficient to enable *those skilled in the art* to practice the invention. A specification is directed to those skilled in the art and need not teach or point out in detail that which is well-known in the art. In re Nelson, 47 CCPA 1031, 280 F.2d 172, 126 USPQ 242 (1960). "Appellant's invention is a new combination of old materials," namely, "a new slitter knife using a well-known carbide cutting blade." The specification discloses that "slitter knives having tungsten carbide blades are currently used."

Affidavits of appellant and Ritz, a metallurgist for more than 35 years who is familiar with metal slitting blades, prima facie show that the terms used in the specification, namely, "tungsten carbide blades" and "tungsten carbide rims," have only one meaning to those skilled in the *cutting* art—a well-known tungsten carbide bonded by an iron group metal. The affidavit of Ritz states:

> that the hard tungsten carbide facing materials referred to in the aforementioned patent application can only consist of hard carbide particles and an iron group metal binder, *usually* cobalt or nickel;
>
> * * *
>
> that * * * a person of ordinary skill in the art of metal slitting * * * would immediately recognize that the "tungsten carbide rim" referred to in the specification is the hard tungsten carbide facing material commonly employed which consists essentially of hard carbide particles bonded by iron group metals * * * . [Emphasis added.]

The record before us prima facie establishes that the terms used in the specification clearly teach those skilled in the metal *slitting* wheel or *cutting* wheel art how to practice appellant's invention; thus, the specification satisfies the requirements of 35 U.S.C. 112.

The solicitor argues that Kistler et al. evidences "the fact that it was known to those in the art that other materials could be successfully used as binders for tungsten carbide particles for use in *cutting* tools." [Emphasis added.] However, the solicitor has fallen into the same error as the board and the

examiner; that is, Kistler et al. relates to resin bonded *abrasive* articles or *grinding* wheels, *not metal slitting knives* to which the instant invention is directed. Although synthetic resin binders for carbide grains may be satisfactory for *abrasive* tools, nothing of record establishes the operability of such binders for rotary slitter knife rim elements. In fact, the record supports the opposite conclusion.

Unduly Broad and Indefinite

[2] We do not consider that the limitation, "a bonded hard metal carbide rim," is unduly broad, and the rejection of claims 16-19 on that ground is untenable. Such a limitation, as evidenced by the record, defines an article which is old and well-known to one skilled in the metal slitting wheel art. Moreover, [3] the claims of a patent are to be construed in the light of the specification and the understanding thereof by those skilled in that art to whom they are addressed. The record further establishes that one skilled in the art here involved would not consider the term "bonded hard metal carbide" broad enough to include resins and ceramics as binders, as well as self-bonded carbide, all apparently unsatisfactory; instead, such a limitation would mean, to one skilled in the art, a carbide bonded with an iron group metal.

The solicitor argues that claims 16-19 omit an essential limitation, namely, an iron group binder, and are therefore, unpatentable for overclaiming the invention. He cites Graver Tank & Mfg. Co., Inc. et al. v. Linde Air Products Co., 336 U.S. 271, 80 USPQ 451 (1949), in support of the proposition that, when the claims improperly overclaim the invention and are free from ambiguity which might justify resort to the specification, they are not to be saved because the latter is less inclusive. However, in the Graver Tank case, involving welding flux, the Supreme Court merely affirmed the District Court's refusal to limit or qualify the claim terms "silicates" and "metallic silicates," so as to mean only the nine specific metallic silicates which had been proved operative and had been disclosed in the specification. In that case, there was no indication that the terms used in the claims would have a more specific meaning to one skilled in the art than the broader *generic* literal meaning; one skilled in *that* art apparently would not have recognized that "silicates" or "metallic silicates" means only the nine metallic silicates disclosed in the specification. Here, however, evidence was introduced which prima facie establishes that "bonded hard metal carbide rim" has but one meaning to those skilled in this art, namely, a hard metal carbide bonded by an iron group metal.

[4] The rejection of claims 16-20 as unduly broad in calling for a supporting member which includes broadly "a powdered metal" is, we feel, likewise untenable. The examiner noted that such limitation "includes the powdered metals of cobalt, nickel, iron and silver * * * which are not taught by appellant as being operative." The board additionally asserted that such highly reactive materials as powdered sodium and lithium would also be included, and the solicitor adds to the list potassium and magnesium which, he suggests, may react with the epoxy resin. Again, the solicitor argues that the claims are unpatentable for overclaiming, citing Graver Tank, supra, and also In re Newton, 38 CCPA 877, 187 F.2d 337, 88 USPQ 554 (1951). However, in claiming a mechanical combination, which the invention here is, an applicant is not necessarily limited to the specific composition which he discloses as the material for making up each and every element of the combination. In re Fuetterer, 50 CCPA 1453, 319 F.2d 259, 138 USPQ 217 (1963). If every element in a mechanical combination claim were required to be so specific as to exclude materials known to be inoperative and which even those *not* skilled in the art would not try, the claims would fail to comply with 35 U.S.C. 112 because they would be so detailed as to obscure, rather than particularly point out and distinctly claim, the invention.

[5] Here, appellant has *described* his invention as *comprehending* the use therein of *any* metal in the supporting member *capable* of performing a specific function in a specific combination, and he has disclosed specifically two such metals having such capability. The examiner and the board, believing that not all metals are capable of performing this function and that one skilled in the art would not know offhand which metals are capable of so functioning, have rejected the claims as unduly broad. But it is clear that the instant claims do not comprehend a class of metals of any greater breadth than is *comprehended* by the invention description which is quoted above under "the invention." It is equally clear from this description and appellant's brief that, in the words of the second paragraph of section 112, "applicant regards as his invention" the slitter knife *combination* with epoxy resin and *any* metal powder *capable* of providing a supporting member having high compressive strength

and resistance to shrinkage. Thus, the limitation of "a powdered metal" is not unduly broad for the reasons expressed in Fuetterer, supra, and in Judge O'Connell's opinion in Newton, supra, where further precedents are cited.

Obviousness

The rejection of claims 16-20 as unpatentable over Van der Pyl in view of Nestor, Kistler et al. and Voegeli-Jaggi under 35 U.S.C. 103 cannot be sustained on the record before us.

The examiner and the board have apparently failed to take into account the precise art to which the invention relates. The invention relates to the sheet metal slitting art wherein a hard metal carbide rim is employed to exert a shearing action. None of the references teaches a hard metal carbide cutting rim and thus none of the references can teach or suggest the expedient of joining a hard metal carbide rim, as that term is used in the prior art and is understood by those skilled in the art, to a sleeve or shaft by means of an epoxy resin and powdered metal supporting member. Combining the references as done by the examiner and sustained by the board [7] will result in an *abrasive* or *grinding* wheel but will not result in appellant's invention, namely, "a rotary slitter knife for slitting metal strip and sheet." In this respect, this case is not unlike Kropa v. Robie et al., 38 CCPA 858, 187 F.2d 150, 88 USPQ 478 (1951), where the claim preamble, "an abrasive article," was held to be a significant limitation which defined a particular article and property of that article and gave "life and meaning to the claim."

New Matter

[6] The rejection of claims 17 and 19 on the basis of new matter in the limitation "no greater than 400" characterizing the epoxide equivalent it reversed because *original* claim 5 [8] clearly supports the terminology in the rejected claims. It is elementary that claims contained in an application as originally filed may be considered part of the disclosure of the application. McBride v. Teeple, 27 CCPA 961, 109 F.2d 789, 44 USPQ 523 (1940).

The rejection of claim 20 on the basis of new matter in the expression "a metal selected from the group consisting of cobalt and nickel" is proper on this record. Although the affidavits of record prima facie establish that "tungsten carbide or other hard metal carbides" means, to one skilled in the art, tungsten carbide or other hard metal carbides bonded by *an iron group* binder, the affidavits do not sufficiently show that such binder is necessarily cobalt or nickel. Appellant's affidavit refers to rim particles as "bonded together with a binder *such as* cobalt or nickel" (emphasis added) and to "cobalt or nickel bonded metal carbides." Certainly the first quoted passage suggests that other binders might be encompassed within the meaning of "tungsten carbide." The affidavit of Ritz mentions "hard carbide particles and an iron group metal binder, *usually* cobalt or nickel," "bonded by an iron group metal *such as* cobalt or nickel," and "hard carbide particles bonded by iron group *metals* [with no examples]." (Emphases added.) It may be inferred from the record that "tungsten carbide" would not necessarily mean *cobalt or nickel* bonded tungsten carbide since other iron group metals may also be suitable. Accordingly, appellant may not specifically claim those two binders after his filing date for want of a specific disclosure even though they may be the most prevalently used.

Conclusion

The decision of the board is *affirmed* as to the rejection of claim 20; the board's affirmance of the rejection of claims 16-19 is *reversed*.

Judge SMITH participated in the hearing of this case but died before a decision was reached.

NEESE, Judge, concurring.

The subject matter of the appellant's invention is in a classification recognized by the Patent Office as cutting wheels,[1] made by a process wherein a hard metal carbide rim, employed to exert a shearing action, is bonded to a hub. The prior art cited by the examiner and relied on by the Board of Appeals relates to abrading wheels.[2]

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[7] The board affirmed the *examiner's* rejection summarized in the text of this opinion, supra, and made no Rule 196(b) rejection.

[8] Original claim 5 which formed a portion of the original disclosure reads:

> 5. A rotary slitter knife comprising a tungsten carbide rim, a hardened steel bushing, and a supporting member between said rim and said bushing and bonding said rim to said bushing, said supporting member consisting essentially of powdered metal and an epoxy resin having an epoxide equivalent of 400 or less.

[1] In Class 33 of the Patent Office Classification Manual entitled "cutting" which includes machines and tools for cutting.

[2] In Class 51 of the Patent Office Classification Manual entitled "abrading" which includes machines and tools for abrading.

I am in accord with Judge Baldwin's opinion that none of these prior art references teaches the expedient of joining a hard metal carbide cutting rim, as that term is used in the prior art and understood by those skilled in the art, to a sleeve or shaft by means of an epoxy resin and powdered metal supporting member; and that a combining of these references will not result in a rotary slitting-knife for slitting metal-strip and -sheet.

The differences between the prior art and the subject matter sought to be patented by the appellant are apparent in the record, i.e., a slitting-wheel which cuts sheet-metal by a shearing action differs in kind from an abrading wheel which removes material by abrasive action; and, in operational use, tungsten carbide is applied in a slitting-wheel, not to remove metal as in abrasion, but to resist wear while providing the force to be imposed in the shearing of sheet-metal.

There is no evidence before us suggesting that a person of ordinary skill in the slitter-wheel art would glean information from the abrading-wheel art in solving this problem in the slitter-wheel art. Thus, under the record before us, the differences between (a) the subject matter sought by the appellant to be patented and (b) the prior art, are not such that such subject matter as a whole would have been obvious at the time the invention was made to a person of ordinary skill in the art to which said subject matter pertained. 35 U.S.C. 103; see Graham v. John Deere Co., 383 U.S. 1, 17, 86 S. Ct. 684, 15 L. Ed. 2d 545, 556 [11], 148 USPQ 459, 466-467 (1966).

For the foregoing reasons, I concur in the reversal of the § 103 rejection, and in all other respects I join the opinion of Judge Baldwin.

WORLEY, Chief Judge, dissenting, with whom ALMOND, Judge, joins.

It seems to me that the majority, in reversing the board's rejection under § 103, has attributed to the board in line of reasoning that it did not really pursue. The majority states:

> * * * None of the references teaches a hard metal carbide cutting rim and thus none of the references can teach or suggest the expedient of joining a hard metal carbide rim, as that term is used in the prior art and is understood by those skilled in the art, to a sleeve or shaft by means of an epoxy resin and powdered metal supporting member. Combining the references as done by the examiner and sustained by the board will result in an *abrasive* or *grinding* wheel but will not result in appellant's invention, namely, "a rotary slitter knife for slitting metal strip and sheet. * * *

I do not think the board intended to imply that it was limiting its consideration solely to what was taught merely to the *abrading* art by a consideration of the various teachings of the references, or that it was relying on that combination alone to render obvious appellant's slitter knife.

It is well settled that we must consider the state of the prior art not only as shown in the references employed, but also as reflected in appellant's specification. In re Davis, 49 CCPA 1196, 305 F.2d 501, 134 USPQ 256 (1962). Here, appellant's specification states:

> * * * Two principal types of slitter knives having tungsten carbide blades are currently used. Slitter knives of small width (⅜" and less) have been made of solid tungsten carbide. Such knives obviously have in them a substantial amount of expensive tungsten carbide which does not serve to perform any of the cutting operation and has to be scrapped or reclaimed after the knife was worn to a certain extent. A tungsten carbide rim is mechanically mounted on a steel hub for knives ½" and wider. In these structures, a screw clamp holds the tunsten carbide rim in position. Although there is less unusable carbide in such structures, the expense of manufacturing and assembling this type is high.

Thus it can be seen that the board, like this court, was faced with a record which establishes beyond peradventure that each element of appellant's claimed combination is old. What is new, insofar as this record shows, is the use of a particular means—a previously known chemical composition consisting essentially of a mixture of epoxy and resin and finely divided metal [1]—to attach

[1] That the epoxy resin-metal powder composition appellant employs was itself known prior to appellant's filing date is clear from certain pages from Lee and Neville "Epoxy Resins" (1957) filed by appellant during proceedings below. Lee points out that epoxy resins "possess a number of unusually valuable properties immediately amenable to use in the formulation of *adhesives*, sealing liquids, *cold solders*, *castings*, laminates, and coatings." [Emphasis supplied.] "Cold solder," in turn, is defined by yet another publication appellant submitted as "composed of finely divided metallic particles dispersed in epoxy resin." Among

the concededly old "bonded hard metal carbide rim" or cutting blade to the equally old "metal bushing" or hub, thereby replacing the clamp holding means previously employed for that purpose in the prior art.

It was in those circumstances that the board stated:

> * * * the references demonstrate that the expedient of joining a hard bonded rim to a sleeve or shaft by means of a thermosetting resin in a powdered metal composition would have been obvious. *The difference in operation between a rotary slitter knife and an abrasive wheel is not a controlling consideration in this case.* The record does *not* suggest that *the problem* of bonding the hardened rim to a supporting sleeve or shaft *is different in these two types of wheels.* [Emphasis supplied.]

The board's language "hard bonded rim" or "hardened rim" is sufficiently broad to encompass either appellant's "bonded hard metal carbide rim" or the abrading rims of the references. It is evident from those references that phenol formaldehyde or epoxy resin materials, with or without metal powder fillers for purposes of heat dissipation, have long been suggested to those in the art as supporting members for abrasive rims. I think the board properly found appellant's particular means for attaching the cutting blade to the hub to be obvious in view of what had been suggested before in the abrading wheel art as shown by the references before us.[2]

I would affirm the § 103 rejection.

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the desirable properties of epoxy resins; Lee mentions "high adhesive strengths," ability "to provide chemical bonds with surfaces, such as metals," and "low shrinkage." Appellant's argument that it "is surprising and unexpected in the art that such a supporting member [of epoxy and powdered metal] would possess the strength, resistance to shrinkage, and adhesive properties requisite for the present application" appears contradicted by Lee.

[2] I agree with the solicitor that:

> * * * The [slitter and abrading] arts would seem, insofar as the supporting structure is concerned, clearly to be analogous. Those in the slitter art would naturally look to abrading wheels for suggestions for improved supporting structures * * *.

Court of Customs and Patent Appeals

In re JURSICH AND RANDICH

No. 8121 Decided May 22, 1969

PATENTS

1. **Construction of specification and claims — By specification and drawings — In general (§ 22.251)**

Double patenting—In general (§ 33.1)

Although disclosure of patent, on which double patenting rejection is predicated, is not available as evidence of prior art against applicants by reason of its later effective filing date, it is proper to refer to patent disclosure to ascertain meaning of expressions employed in claims.—In re Jursich & Randich (CCPA) 161 USPQ 675.

2. **Double patenting — In general (§ 33.1)**

Although applicants are not claiming same invention as that claimed in patent issued on copending application to common assignee, claims are rejected for double patenting since process claimed by applicants is but an obvious variation, in double patenting rather than 35 U.S.C. 103 sense, of process claimed in patent.—In re Jursich & Randich (CCPA) 161 USPQ 675.

3. **Court of Customs and Patent Appeals — Issues determined — Ex parte patent cases (§ 28.203)**

Disclaimer—Time to disclaim (§ 32.8)

Court cannot consider terminal disclaimer filed after Board's decision where Board refused to consider disclaimer because it was not timely presented or considered by examiner.—In re Jursich & Randich (CCPA) 161 USPQ 675.

Particular patents—Paper Making

Jursich and Randich, Polymeric Compositions and Their Use in Paper Making Process, claims 1 to 5 of application refused.—In re Jursich & Randich (CCPA) 161 USPQ 675.

Appeal from Board of Appeals of the Patent Office.

Application for patent of Myron J. Jursich and Gail T. Randich, Serial No. 398,418, filed Sept. 22, 1964; Patent Office Group 170. From decision rejecting claims 1 to 5, applicants appeal. Affirmed; Baldwin, Judge, concurring with opinion.

HERBERT B. KEIL and MARZALL, JOHN-

the parties and was unlawful and against public policy.

While certain parts of plaintiff's obligations under the contract were legal, standing alone, those parts are inextricably intertwined with the illegal agreement to disclose the Harris process, and for that reason the entire consideration for the note must be considered illegal and void. In Bourland v. First National Bank Building Co., 152 Ark. 139, 149, 237 S.W. 681, it was said:

> "It is well settled that if any part of the entire consideration for a promise or any part of an entire promise be illegal, whether by statute or by the Constitution or from considerations of public policy, the whole contract is void. * * *
>
> "Where there are provisions in a contract for a compensation which is legal, still if they are blended with those which are forbidden, the whole is a unit and indivisible. The above is the language of Mr. Justice Swayne in Trist v. Child, 21 Wall. (U.S.) 441. The learned justice added that that which is bad destroys that which is good, and they period together.
>
> "Where the lawful and unlawful parts of a contract can not be separated so as to enforce the one and annul the other, it is an indivisible contract and therefore null and void throughout. Edwards v. Randle, 63 Ark. 318. And in that case the court quoted with approval the following:
>
> "'If any part of an indivisible promise, or any part of an indivisible consideration for a promise, is illegal, the whole is void.'"

It should be remembered that the plaintiff has already received $8,050 from the defendant, and to permit the plaintiff to recover in this case would be to allow him to recover still another $10,000 as the fruits of his theft of his employer's secrets. The Court is unwilling to countenance such a result.

Nor does the defendant stand before the Court in the robe of the innocent. On the contrary, the Court considers him just as culpable as the plaintiff. It was defendant who conceived the plan of luring Williams away from the employ of Harris for the purpose of starting a competing business. Defendant's plan at best smacks of the unethical. The Court is convinced that whether or not defendant knew that plaintiff was going to steal the Harris process, he strongly suspected that such would be done and expected it to be done; and when dissolution of the partnership was agreed upon, the defendant proposed to obtain the secret from the plaintiff and to carry on the unlawful operation. In the Court's opinion, the parties are in pari delicto, and the defendant's counterclaim should be dismissed along with the plaintiff's complaint.

It is, therefore, by the Court considered, ordered, and adjudged that the complaint be, and the same hereby is, dismissed; that the defendant's counterclaim be, and the same hereby is, dismissed; and that each party shall bear his own costs.

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47 CCPA 787

Court of Customs and Patent Appeals

In re GRIMME, KEIL, and SCHMITZ

Appl. No. 6475 Decided Feb. 9, 1960

PATENTS

1. **Interference—Reduction to practice—Constructive reduction (§ 41.755)**

Since earlier application on which applicants' parent patent was granted was copending with instant continuation-in-part application and was filed prior to issuance of applicants' Belgian patent, claim cannot be rejected on Belgian patent if earlier application contained disclosure sufficient to support claim.

2. **Court of Customs and Patent Appeals—Record (§ 28.30)**

While applicants' earlier application on which issued its parent patent is not included in court record, it is presumed, in absence of contrary showing, that its disclosure corresponds to parent patent, a copy of which is in record.

3. **Specification—Sufficiency of disclosure (§ 62.7)**

In considering sufficiency of disclosure to support generic or subgeneric claim in field of chemistry, court has consistently held that naming of one member of such a group is not, in itself, proper basis for claim to entire group; however, it may not be necessary to enumerate plurality of species if genus is sufficiently identified in application by other appropriate language; what constitutes such language depends on circumstances of each case; where claimed group involves compounds which differ radically from each other, it may not be sufficient to identify group broadly and to name one or two compounds falling within it; on the other hand, in the case of a small and closely related group such as the halogens, naming of group should ordinarily be sufficient since nothing of consequence would be added by also naming each of the well known members of group.

4. Specification—Sufficiency of disclosure (§ 62.7)

Claimed compounds form subgenus under genus sufficiently disclosed in applicants' parent application to support allowable generic claim; scope of subgenus is indicated by statement that compounds might comprise "a piperidine ring," and one example of compound containing such a ring is given, together with eight examples of similar compounds not including such a ring; disclosure is sufficient; parent application's disclosure expressly points out that piperidino salts form definite part of applicants' generic invention, and gives one example of such a compound together with number of other examples of compounds included in genus; instant piperidino compounds do not differ radically from each other or from compounds claimed in parent patent; hence, examples are adequate to show those skilled in art how invention of subgenus claims is to be practiced; it is noted that four added examples in instant application involve compounds and procedures similar to those of original example; added examples, therefore, involve only what could have been readily ascertained by those skilled in art on basis of application disclosure.

5. Specification—Sufficiency of disclosure (§ 62.7)

It is impracticable for applicant who discloses generic invention to give example of every species falling within it or even to name every such species; it is sufficient if disclosure teaches those skilled in art what the invention is and how to practice it.

Particular patents—Penicillin

Grimme, Keil, and Schmitz, Penicillin Salts of Amino Salicylates, claim 1 of application allowed.

Appeal from Board of Appeals of the Patent Office.

Application for patent of Walter Grimme, Werner Keil, and Heinrich Schmitz, Serial No. 453,408, filed Aug. 31, 1954; Patent Office Division 6. From decision rejecting claim 1, applicants appeal. Reversed.

BURGESS, DINKLAGE & SPRUNG (ARNOLD SPRUNG of counsel) both of New York, N.Y., for appellants.

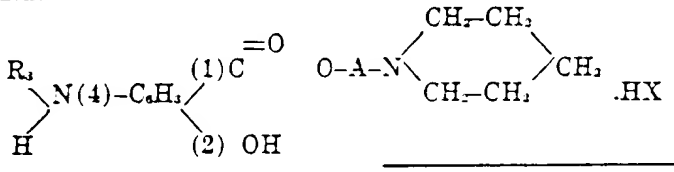
CLARENCE W. MOORE (J. SCHIMMEL of counsel) for Commissioner of Patents.

Before WORLEY, Chief Judge, RICH, MARTIN, and SMITH, Associate Judges, and KIRKPATRICK, Judge.*

WORLEY, Chief Judge.

This appeal is from the decision of the Board of Appeals of the United States Patent Office affirming the rejection by the Primary Examiner of the single claim of appellants' application for a patent on penicillin salts of amino salicylates. The appealed claim reads:

1. As a new chemical compound, a penicillin salt of an amino-salicylate having the general formula:



in which HX is penicillin in the form of its acid: R_1 is a radical containing not in excess of 10 carbon atoms and selected from the group consisting of hydrogen, alkyl, and phenylalkyl radicals: and A is a saturated aliphatic hydrocarbon radical having 2 to 5 carbon atoms.

The following references were relied on by the examiner:

Goldberg et al., 2,493,625, January 3, 1950.

Goldman, 2,547,640, April 3, 1951.

Grimme et al., 2,701,796, February 8, 1955.

Gewerkschaft etc. (Belgian), 506,999, November 30, 1951.

The application was filed August 31, 1954, and is a continuation in part of application No. 255,748, filed November 9, 1951, on which patent No. 2,701,796 was granted February 8, 1955. Appellants were granted a Belgian patent on November 30, 1951, having a disclosure corresponding to that of their earlier United States application. That patent was published March 27, 1953, more than a year prior to the filing of the appealed application.

The examiner rejected the claim primarily on the ground that it was drawn to the invention claimed in appellants' patent No. 2,701,796, thus its allowance would result in double patenting; and, alternatively, that if the claim was drawn

* United States Senior District Judge for the Eastern District of Pennsylvania, designated to participate in place of Judge O'CONNELL, pursuant to provisions of Section 294(d), Title 28, United States Code.

to a different invention then such invention was not supported by the copending application on which the patent was granted, thus allowance of the claim was barred by the Belgian patent.

The board reversed the rejection based on double patenting, but affirmed on the Belgian patent. Accordingly, only the latter ground of rejection is before us.

[1] Since the earlier application on which appellants' patent No. 2,701,796 was granted was copending with the one on appeal and was filed prior to issuance of the Belgian patent it is evident that if such earlier application contained a disclosure sufficient to support the appealed claim the rejection of that claim was improper (35 U.S.C. 120). While the earlier application has [2] not been included in the record, it is presumed, in the absence of a showing to the contrary, that its disclosure corresponds to that of patent No. 2,701,796, a copy of which appears in the record.

While the formula set forth in the claim is complex, the issue here concerns primarily the piperidine ring, i.e. the combination of the "N" and the CH_2 groups in the right-hand portion of the formula and, accordingly, the remainder of the formula need not be considered in detail.

Appellants' earlier application discloses a broad group of penicillin salts of amino salicylates having formulae generally similar to that of the appealed claim. In most of the compounds disclosed the attachments to the "N" in the right-hand portion of the formula consist of alkyl, aryl, aryl alkyl or alkyl aryl groups, but the specification states that the attachments, together with the "N" may also be a heterocyclic ring, as, for example, "a piperidine ring." The appealed claim is directed to compounds of the piperidine type, while the claims of the patent granted on the earlier application are drawn to those which include aryl and alkyl groups in lieu of the piperidine ring.

It is evident from the disclosure of their earlier application that appellants regarded the compounds of the appealed claim and those claimed in patent No. 2,701,796 as constituting a single generic invention, and it was stated by the examiner in his final rejection in the instant case that an allowable claim generic to all those compounds could have been formulated. In other words, the compounds claimed here represent a subgenus under a patentable genus which was sufficiently disclosed in appellants' earlier application. The application gives nine examples coming within the genus, only one of which involves a piperidine ring.

The basis for the rejection is summed up in the following statement in the board's decision:

> We do not consider this single example [the one last referred to above] as sufficient disclosure to support the number of compounds encompassed by the broad claim on appeal.

The board pointed out that the instant application discloses not only the example of the earlier application, but also four additional examples of compounds falling within the scope of the appealed claim. Since no question has been raised as to the sufficiency of that disclosure, it appears that the Patent Office tribunals were of the opinion that the invention of the appealed claim was sufficiently delineated by five examples but not by one.

[3] The question as to the sufficiency of disclosure to support a generic or subgeneric claim in the field of chemistry has frequently been considered by this court and it has been consistently held that the naming of one member of such a group is not, in itself, a proper basis for a claim to the entire group. In re Steenbock, 23 CCPA 1244, 83 F.2d 912, 30 USPQ 45; In re Kyrides, 34 CCPA 920, 159 F.2d 1019, 73 USPQ 61; and In re Soll, 25 CCPA 1309, 97 F.2d 623, 38 USPQ 189. However, it may not be necessary to enumerate a plurality of species if a genus is sufficiently identified in an application by "other appropriate language." In re Dreshfield, 27 CCPA 1013, 110 F.2d 235, 45 USPQ 36; In re Oppenauer, 31 CCPA 1248, 143 F.2d 974, 62 USPQ 297.

What constitutes "other appropriate language" within the meaning of the cited cases will, of course, depend on the circumstances of each particular case. Where the claimed group involves compounds which differ radically from each other it may not be sufficient to identify the group broadly and to name one or two compounds falling within it. In re Cox, 40 CCPA 720, 198 F.2d 346, 95 USPQ 94, and cases there cited. On the other hand, in the case of a small and closely related group such as the halogens, the naming of the group should ordinarily be sufficient since nothing of consequence would be added by also naming each of the well known members of the group.

[4] In the instant case, as above noted, the claimed compounds form a subgenus under a genus which the examiner found to have been sufficiently disclosed in the earlier application to support an allowable generic claim. The scope of the subgenus was indicated by

the statement that the compounds might comprise "a piperidine ring," and one example of a compound containing such a ring was given, together with eight examples of similar compounds which did not include such a ring.

[5] In our opinion that disclosure is sufficient. It is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species. It is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it. Here the parent application disclosure expressly points out that piperidino salts formed a definite part of appellants' generic invention, and gives one example of such a compound together with a number of other examples of compounds included in the genus. There is nothing to show that the piperidino compounds involved here differ radically from each other or from the compounds claimed in patent No. 2,701,796. It would appear, therefore, that the examples given are adequate to show those skilled in the art how the invention of the appealed claims is to be practiced. It is to be noted that the four added examples in the instant application, which the examiner and the board found sufficient, coupled with the original example of a piperidine ring compound, to support the appealed claim, involve compounds and procedures quite similar to those of the original example. The added examples, therefore, appear to involve only what could have been readily ascertained by those skilled in the art on the basis of the application disclosure.

Our conclusion that the disclosure of appellants' earlier application is sufficient to support the appealed claim requires reversal of the decision of the Board of Appeals.

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47 CCPA 797

Court of Customs and Patent Appeals

In re TROIEL

Appl. No. 6480 Decided Feb. 9, 1960

PATENTS

1. Patentability—Aggregation or combination—Of old elements (§ 51.159)

Combination of well known mechanical features which function no differently than one skilled in art would expect is not patentable invention.

2. Patentability—New use or function—In general (§ 51.551)

Patentability of mechanical claims cannot be based upon process with which apparatus is to be used.

3. Patentability — Change — Size or strength (§ 51.261)

Mere change of relative size of coacting members of known combination will not endow otherwise unpatentable combination with patentability.

Particular patents—Hole Cap

Troiel, Hole Cap and Tool Therefor, claims 2, 4, 5, and 10 of application refused.

Appeal from Board of Appeals of the Patent Office.

Application for patent of Arthur E Troiel, Serial No. 165,039, filed May 29, 1950; Patent Office Division 66. From decision rejecting claims 2, 4, 5, and 10, applicant appeals. Affirmed.

BRUCE & BROSLER, Berkeley, Calif., for appellant.

CLARENCE W. MOORE (S. WM. COCHRAN of counsel) for Commissioner of Patents.

Before WORLEY, Chief Judge, RICH, MARTIN, and SMITH, Associate Judges, and KIRKPATRICK, Judge.*

MARTIN, Judge.

This appeal is from the decision of the Patent Office Board of Appeals affirming the final rejection of claims 2, 4, 5 and 10, the only claims remaining in application Serial No. 165,039, filed May 29, 1950, entitled "Hole Cap and Tool Therefor."

In setting up forms into which concrete is poured for making structures such as walls, dams, etc., oft times, either initially or in reuse, the lumber used has holes or other imperfections in its surfaces. To prevent the concrete, as it is poured into the forms, from escaping through holes and to provide a smooth surfaced concrete structure, something is needed to cover the holes or other defects in the wood. It is to this that appellant's invention is directed.

Appellant denominates that which is used for covering the holes a "hole cap." The cap itself is a disk having, on a circle concentric with and inwardly of the periphery of the disk, a plurality of tabs triangular in shape pushed out to extend from one side only of the disk.

* United States Senior District Judge for the Eastern District of Pennsylvania, designated to participate in place of Judge O'CONNELL, pursuant to provisions of Section 294(d), Title 28, United States Code.

APPENDIX D

Liang et al. (Gene, 80, (1989) 21-28))

Gene, 80 (1989) 21–28
Elsevier

GENE 03061

Efficient cloning of a mutant adenylate-kinase-encoding gene from *Escherichia coli*

(Recombinant DNA; *adk*; homogenotization; temperature-sensitive mutation; low-copy-number plasmid)

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SUMMARY

An optimized system has been developed for the transfer of a mutant gene from the *Escherichia coli* chromosome to a plasmid carrying the wild type (wt) allele. The wt allele was first cloned into a low-copy-number, self-transmissible plasmid with a single *Eco*RI, *Hin*dIII, and *Bam*HI site. The plasmid was then transferred to a mutant strain that had been previously transformed with a high-copy-number plasmid carrying the *recA*$^+$ gene to allow efficient homologous recombination. A 15% frequency of homogenotization was obtained during cloning of an *adk* gene that encodes a temperature-sensitive adenylate kinase (AK). The mutant AK had decreased mobility on sodium dodecyl sulfate–polyacrylamide gels compared with the wt enzyme. This was due to a point mutation that changed leucine-107 in the wt enzyme to glutamine-107 in the mutant enzyme as determined by nucleotide sequencing.

INTRODUCTION

Adenylate kinase (AK; EC 2.7.4.3) is ubiquitous in living organisms. The enzyme catalyzes an interconversion of adenine nt, MgATP + AMP ⇄ MgADP + ADP, and studies with temperature-sensitive mutants in *E. coli* have shown that AK is an essential enzyme. The *adk* mutants have been shown to have a very complex phenotype. Some of these mutants were originally isolated as being defective in phospholipid synthesis (Cronan et al., 1970), while others were initially isolated as *dnaW* mutants based on their inability to effect conjugal DNA transfer at the nonpermissive temperature (Henson et al., 1982). Temperature-sensitive mutants defective in AK also have been isolated by Cousin et al. (1967; 1969).

Complementation analysis showed that all these mutations were in the structural gene for AK (Esmon et al., 1980). At the nonpermissive temperature there are changes in adenine nt concentrations that cause

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Abbreviations: aa, amino acid(s); *adk*, gene encoding AK; AK, adenylate kinase; Ap, ampicillin; bp, base pair(s); kb, kilobase(s) or 1000 bp; Km, kanamycin; LB, Luria–Bertani (medium); MC, mitomycin C; Nal, nalidixic acid; nt, nucleotide(s); ^{R}, resistance; SDS, sodium dodecyl sulfate; Sm, streptomycin; Tc, tetracycline; ts, temperature sensitive; wt, wild type; ::, novel joint (insertion); [ ], designates plasmid-carrier state.

a coordinate decrease in all macromolecular biosynthesis and a cessation of growth (Glaser et al., 1973; 1975).

The wt *adk* gene has recently been cloned and sequenced by this laboratory as well as by other laboratories (Brune et al., 1985; Gilles et al., 1986; Girons et al., 1987). The enzyme consists of 214 aa residues deduced from nucleotide sequence analysis and confirmed by amino acid sequence analysis (Gilles et al., 1986; Girons et al., 1987). Since all the *adk* mutants have thermolabile AKs and some have been found to have changed kinetic parameters, it is of interest to determine the nature of different mutations to gain a better insight into how the enzyme functions. One recent achievement was the identification of a single aa change from Pro-87 to Ser-87 that causes AK to become thermolabile (Gilles et al., 1986). This was done by protein purification and peptide sequence analysis. In this paper we report a more simple and efficient way to determine the type of mutation in AK, and its potential application to study any mutant bacterial gene.

Often when a wt gene is available, cloning of mutant alleles is done by in vivo homologous DNA recombination (Stauffer et al., 1979; Horwitz et al., 1980). This method, though theoretically simple, has its limitations especially since the frequency of homogenotization can be lower than 0.02% when applied to small segments of DNA cloned into conventional high-copy-number plasmid vectors (Horwitz et al., 1980; Chattoraj et al., 1984). Screening of thousands of colonies is necessary to recover a few desired mutant phenotypes which is laborious if no positive selection is available. This report describes an approach which substantially increases the frequency of homogenotization and permits the identification of the ts homogenotes.

MATERIALS AND METHODS

(a) Materials

Antibiotics were obtained from the Sigma Chemical Company, St. Louis, MO. Restriction endonucleases and T4 DNA ligase were purchased from BRL, Gaithersburg, MD, and New England Biolabs, Beverly, MA, respectively. The Vector Stain ABC Kit for Western-blot analysis was obtained from Vector Lab., Inc., Burlingame, CA. Nitrocellulose filter discs were obtained from Schleicher & Schuell, Inc., Keene, NH.

(b) Bacterial strains, plasmids, and growth conditions

The bacterial strains and plasmids used in this study are listed in Table I. All *E. coli* strains are K-12 derivatives. Minimal medium used for phage Mu-mediated transposition of the *adk*$^+$ gene was described previously (Esmon et al., 1980). Otherwise all strains were routinely grown in LB-broth (Maniatis et al., 1982).

(c) Isolation and manipulation of DNA

Both low-copy-number and high-copy-number plasmids were isolated by the miniprep method of Maniatis et al. (1982). For DNA sequencing, plasmids were prepared by CsCl-density-gradient centrifugation (Maniatis et al., 1982). Restriction enzyme digestions and ligations of DNA were carried out as instructed by the suppliers. Other routine procedures for molecular biology were carried out as described in Maniatis et al. (1982).

(d) In vivo cloning of the *adk*$^+$ gene

Phage Mu-mediated transposition of the *adk*$^+$ gene was done as described by Mendoza et al. (1981) except RP4 instead of R6K was used as the recipient plasmid (Barth and Grinter, 1977). For the conjugative transfer of the plasmid, the donor strain (RG144) and the recipient strain KG10 (*adk*-6) that had been made into a Muc$^+$ lysogen (Faelen and Toussaint, 1976), were grown in LB-broth medium overnight at 30°C. The donor was diluted 100-fold in fresh LB-broth and grown with good aeration at 37°C for 4 h to partially induce the Mucts prophage. The recipient was diluted 50-fold and grown at 30°C for the same period of time. Equal volumes of donor and recipient were mixed for 3 h at 37°C to allow conjugation. Cells were then washed with minimal medium and spread onto selective plates at 40°C. Transconjugants were isolated by selection for Ap resistance of RP4 and complementation of the ts phenotype of the *adk* mutant. The donor was

TABLE I

Bacteria and p

Strain or plasmid	
E. coli:	
KL218	
-ul	
KG10	
KG3	
CV2	
RG144	
bn	
JM83	
LP1 to LP5	
LP8	
LP11 to LP1	
LP101	
LP105	
LP202	
Plasmids:	
RP4	
pUC13, pUC	
pBR322	
pLP3	
pLP315	
pLP315-C	
pLC24-31	
pLC21-33	
pLP315-C*	
pLP105	
pLP101	
pLP202	

counterse
tryptophar
was lethal
Mucts-62

(e) In vivo

KG10 (
either pL(
mids carr
Carbon b
Neidhardt
plasmid v
et al., 197
*Hin*dIII fr
low-copy-
RP4) was

TABLE I

Bacteria and plasmids

Strain or plasmid		Genotype	Source
E. coli:			
KL218	F⁻	*proC*24 *purE*41 *thyA*25 *nalA*12 *argG*34 *metB*1 *his*-53 *pyrC*30 *lac str*-97 *tsx*-63 *mlt*-2 *xyl*-7 or 14	Esmon et al. (1980)
KG10	F⁻	*adk*-6 *purE*⁺ *his*⁺ *recA*1 Nal^S other markers as in KL218	Esmon et al. (1980)
KG3	F⁻	*adk*-31 *purE*⁺ *his*⁺ *recA*1 Nal^S other markers as in KL218	Esmon et al. (1980)
CV2	Hfr	*adk*-2 *glpD*3 *glpR*2 *phoA*8 *tonA*22 $T2^R$ *rel*-1 (λ)	Cronan et al. (1970)
RG144		[RP4 (Ap^R Tc^R Km^R), pBC4042 (Mucts-62 Km^R ColE1 min mud)] Δ(*argF-lacIPOZYA*)U169 *trp* Δ(*brnO-phoA-proC-phoB -phoR*) *nadA*::Tn*10* *cyd* Sm^R	D. Au and R.B. Gennis
JM83		*ara* Δ(*lac-pro*) *thi* (φ80d*lacZ*ΔM15)	Vieira and Messing (1980)
LP1 to LP5		KG10[Mucts/*adk*⁺::RP4]	This work
LP8		KG10[pLP315-C/*adk*⁺]	This work
LP11 to LP14		KG10[pLP315-C*/*adk*-6]	This work
LP101		KG10[pLP101]	This work
LP105		KG10[pLP105]	This work
LP202		KG10[pLP202]	This work
Plasmids:			
RP4		Ap^R Tc^R Km^R Tra^+	Barth and Grinter (1977)
pUC13, pUC19		Ap^R	Vieira and Messing (1982)
pBR322		Ap^R Tc^R	Bolivar et al. (1977)
pLP3		Mucts/*adk*⁺::RP4	This work
pLP315		Ap^R Tc^R Tra^+ RP4 origin	This work
pLP315-C		13-kb fragment C/*adk*⁺ in pLP315 *Hin*dIII site	This work
pLC24-31		ColE1 *recA*⁺	Neidhardt et al. (1983)
pLC21-33		ColE1 *recA*⁺	Neidhardt et al. (1983)
pLP315-C*		13-kb fragment C/*adk*-6 in pLP315 *Hin*dIII site	This work
pLP105		13-kb fragment C/*adk*⁺ in pUC13 *Hin*dIII site	This work
pLP101		a 1.4-kb *Cla*I fragment containing *adk*⁺ in pBR322	This work
pLP202		13-kb fragment C/*adk*-6 in pUC19 *Hin*dIII site	This work

counterselected by plating on medium without tryptophan, as well as by the high temperature which was lethal as a result of the full induction of the Mucts-62 prophage (Faelen and Toussaint, 1976).

(e) In vivo cloning of the *adk*-6 gene

KG10 (*adk*-6, *recA*1) was first transformed with either pLC24-31 or pLC21-33, recombinant plasmids carrying the *recA*⁺ gene from the Clarke–Carbon bank of *E. coli* (Clarke and Carbon, 1976; Neidhardt et al., 1983). KG10 containing a *recA*⁺ plasmid was selected with 0.5 μg MC/ml (Lloyd et al., 1974). The *adk*⁺ gene cloned on a 13-kb *Hin*dIII fragment (fragment C) in pLP315 (a 28-kb low-copy-number, conjugative plasmid derived from RP4) was readily transformed into JM83. The plasmid was subsequently transferred by conjugation to KG10 containing a *recA*⁺ plasmid. KG10 containing both plasmids was selected on plates containing (per ml) 100 μg Sm, 0.5 μg MC and 25 μg Tc at 30°C. The donor was counterselected with Sm, whereas Tc was used to select the presence of pLP315-C and MC was used to select either pLC24-31 or pLC21-33 as well as induction of the *recA*⁺. KG10 with the two plasmids was randomly restreaked on Sm, MC, and Tc plates at 30°C. Cells were scraped from the surface of the plate and resuspended in 5 ml LB-broth containing Tc. Dilutions ($1:10^4$ and $1:10^5$) of the culture were made and 500 μl were spread onto a nitrocellulose filter disc which had been placed on Sm/Tc plates (150 × 15 mm). The plates were incubated at 30°C for approx. 14 h until tiny colonies were visible (pin-

point size). A replica of the colonies was made on a new nitrocellulose filter disc and placed on an Sm/Tc plate. The master plate was further incubated at 30°C, whereas the replica plate was incubated at 40°C. The ts colonies were identified directly from the 40°C plate as light spots (no growth) and recovered from the master plate.

(f) Western-blot analysis

Samples for Western-blot analysis were prepared from 10 ml overnight cultures grown in LB-broth. The cultures were centrifuged in a clinical centrifuge for 10 min and the cell pellets were resuspended in 1 ml of 50 mM phosphate buffer pH 7.0. The cells were broken by using a Heat Systems sonicator model W-375 for 30 s at an output scale of 2.5 and the homogenates were centrifuged in an eppendorf centrifuge for 10 min to remove cell debris. Protein (50 μg) from each extract was analyzed on a Western blot as described by Burnette (1981). The Vector Stain ABC Kit was employed for immunostaining. Antiserum to AK from *E. coli* was prepared in a New Zealand white female rabbit. Purified AK (5 mg) in 1 ml of 50 mM Tris · Cl pH 8.0 was mixed with an equal volume of Freund's complete adjuvant. Aliquots (0.1 ml) of the emulsion were injected intradermally at multiple sites. Six weeks later the rabbit was boosted with 3 mg of purified AK in incomplete Freund's adjuvant and antiserum was collected one week after the booster. Crude antiserum was used without further purification.

(g) Sequencing of DNA

Supercoiled double-stranded plasmid DNA was used as templates for Sanger dideoxy sequencing (Zagursky et al., 1985), except that [^{32}P]dATP was replaced with [^{35}S]dATP. Primers, 14–15 nt long, were made by the Biotechnology Center, Genetic Engineering Facility of the University of Illinois.

RESULTS AND DISCUSSION

(a) Mu-mediated transposition of the *adk*$^+$ region

Faelen and Toussaint (1976) have devised a method to clone genes from *E. coli* or related bacteria. They have shown that chromosomal genes are transposed to conjugative plasmids after heat induction of the Mucts-62 prophage. Plasmids complementing any selectable chromosomal marker can be isolated by transferring the plasmid into an appropriate recipient strain. This is an easy way to obtain an enriched source of specific chromosomal DNA segments for subsequent in vitro cloning. Mu-mediated transposition was carried out with RG144 which contains the Mucts-62 prophage, the wt *adk* gene, and the plasmid RP4. An overnight culture grown at 30°C was diluted 100-fold in LB-broth and shifted to 37°C for 4 h to partially induce the prophage without causing lysis of the cells. The plasmid was transferred to KG10, a *recA*1, *adk*-6 ts mutant that had been made lysogenic with wt Mu to prevent zygotic induction. Five temperature-resistant transconjugants were examined and they all contained the plasmid with the wt *adk* gene. They were designated LP1, LP2, LP3, LP4, and LP5. The plasmids in the five temperature-resistant colonies were additionally shown to be able to complement Adk ts phenotypes by subsequent conjugation into an *adk*-5, Nal^R strain, as well as to confer resistance to Ap, Tc, and Km. One of the plasmids, pLP3, was used for subcloning of the *adk*$^+$ gene.

(b) Subcloning of the *adk*$^+$ gene

Complete *Hin*dIII digestion of pLP3 gave more than six fragments that were each larger than 10 kb. The digest was self-ligated and used to transform KG10. Two plasmids of interest were recovered that conferred resistance to Ap and Tc. The smallest of the two (28 kb), designated pLP315, contained a single *Hin*dIII site and did not complement the *adk*-6 ts gene of KG10. The second plasmid, designated pLP315-C, was made up of pLP315 with a 13-kb *Hin*dIII insert (fragment C) that carried the *adk*$^+$ gene. KG10 (*adk*-6) produced a mutant AK with lower mobility on SDS–polyacrylamide gels whereas other *adk* mutants [e.g., KG3 (*adk*-31) and CV2 (*adk*-2)] produced a ts AK with the same mobility as the wt enzyme (Fig. 1). The introduction of pLP315-C into KG10 led to approx. a threefold overproduction of the wt AK in the mutant background (Fig. 1, lanes 3 and 4), indicating the presence of *adk*$^+$ on the plasmid. Subsequent subcloning of the 13-kb *Hin*dIII fragment into pUC13

Fig. 1. Western-blot
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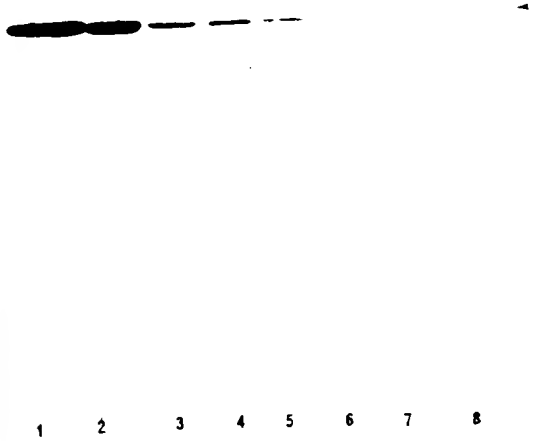

Fig. 1. Western-blot analysis of adenylate kinases. Of total protein 50 μg were analyzed from crude extracts of each strain. Lanes: 1, LP101 (*adk*$^+$ on pBR322 in KG10); 2, LP105 (*adk*$^+$ on pUC13 in KG10); 3, LP8 (*adk*$^+$ on pLP315 in KG10); 4, LP3 (*adk*$^+$ on RP4 in KG10); 5, KL218 (*adk*$^+$); 6, CV2 (*adk*-2); 7, KG3 (*adk*-31); 8, KG10 (*adk*-6) (see Table I). The arrowhead indicates the mutant AK from KG10 (in lanes 1–4 and 8). Note that the other mutant AKs (lanes 6 and 7) have the same mobility as the wt AK (lane 5). Also note the *adk*$^+$ gene cloned on the low-copy-number plasmid (pLP315-C) was expressed in KG10 (lanes 3 and 4) and was greatly overproduced when cloned into high-copy-number plasmid vectors (lanes 1 and 2).

(designated pLP105) and a further trimming to a 1.4-kb *Cla*I fragment into pBR322 (designated pLP101) led to approx. a 60-fold overproduction of the wt AK (Fig. 1, lanes 1 and 2).

(c) Cloning of the *adk*-6 gene

Cloning of the mutant gene is greatly simplified once the wt allele has been cloned (Stauffer et al., 1979; Horwitz et al., 1980; Chattoraj et al., 1984; Chang and Cronan, 1985). When a plasmid carrying a wt gene is placed in a host containing a mutant allele, homogenotization usually occurs at low frequency. It is *recA*-dependent since it involves homologous genetic recombination (Clark, 1973). To transfer a mutant *adk* gene onto pLP315-C, a plasmid carrying the *recA*$^+$ gene (either pLC24-31 or pLC21-33) was first used to transform KG10 (*adk*-6) followed by introduction of pLP315-C through conjugation. The presence of both plasmids in KG10 was selected by growth on plates containing MC (for the *recA*$^+$ plasmid) and Tc (for pLP315-C) and confirmed by the pattern of restriction enzyme digestion (not shown). After restreaking the cells on the plates containing MC and Tc at 30°C to allow homologous genetic recombination to proceed, the cells were resuspended in LB-broth containing Tc. Serial dilutions were made and the cells were replica-plated on nitrocellulose filter discs. Out of 440 colonies, 64 ts colonies were scored on a single plate, a frequency of 15% homogenotization. The ts colonies were very easily identified directly on the replica at the non-permissive temperature as residual cells (non-growing cells). The ts candidates were restreaked on plates with Sm and Tc to select KG10 containing pLP315-C* (now carrying the *adk*-6 gene instead of the *adk*$^+$ gene). Restriction analysis showed that the *recA*$^+$ plasmid was lost without the MC selection which made isolation of pLP315-C* easier (not shown).

(d) Analysis of the *adk*-6 gene product on Western blots

The *adk*-6 gene product, a ts AK, has a slower mobility on SDS–polyacrylamide gels, providing a method to differentiate the mutant enzyme from the wt enzyme. Four ts isolates (designated LP11 to LP14) of KG10[pLP315-C*] were examined. They showed a few-fold overproduction of the mutant AK with no trace of the wt enzyme as expected (Fig. 2, lanes 2 to 5). Two randomly picked temperature-resistant colonies that arose during the isolation of KG10[pLP315-C*] were examined as well. One had a few-fold more wt enzyme than mutant enzyme, as expected for KG10[pLP315-C] that had not undergone recombination and homogenotization, so the wt gene remained on the plasmid and the mutant gene remained on the bacterial chromosome (Fig. 2, lane 8). The other temperature-resistant colony, however, was just the opposite with a few-fold more mutant enzymes than wt enzymes (Fig. 2, lane 7). This is consistent with having the wt gene on the chromosome and the mutant gene on the plasmid which could be an intermediate in the homogenotization process.

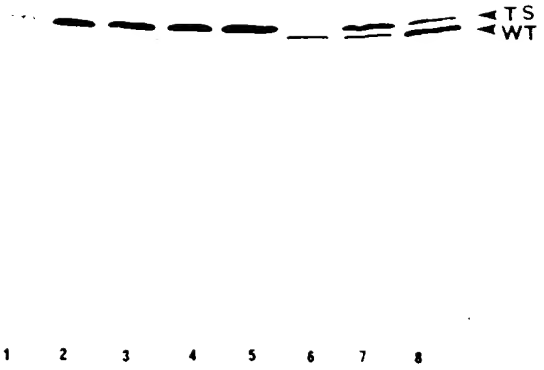

Fig. 2. Western-blot analysis of AK from strains carrying *adk*-6 plasmids obtained from replica plating. Of total protein 50 μg from each crude extract were analyzed. Lanes: 1, KG10 (*adk*-6); 2 to 5, LP11 to LP14 (KG10[pLP315-C*/*adk*-6]); 6, KL218 (*adk*$^+$); 7, KG10[pLP315-C*/*adk*-6] with *adk*-6 on the chromosome replaced by *adk*$^+$ as a result of homogenotization; 8, KG10[pLP315-C/*adk*$^+$]. TS stands for the ts mutant enzyme and WT stands for the wt enzyme.

(e) Subcloning of the *adk*-6 gene

The pLP315-C* plasmid carrying the *adk*-6 gene was isolated from LP12 and found to have the same restriction enzyme pattern as the original plasmid carrying the wt gene, indicating homologous DNA recombination (not shown). The 13-kb *Hin*dIII fragment (C*) was then inserted into the *Hin*dIII site of pUC19 which could be used to produce very large amounts of the mutant AK (not shown). The resulting plasmid, designated pLP202, was used as a template for nucleotide sequencing.

(f) Nucleotide sequence analysis

The sequencing covered the complete coding region along with the 5′ and 3′ flanking regions of the *adk*-6 gene. One point mutation was found in the *adk*-6 gene and this was an A-T to T-A transversion in the middle of the gene to give an aa substitution of Gln-107 for Leu-107 (Fig. 3).

(g) Conclusions and discussion

Mutations in the *adk* gene of *E. coli* are particularly interesting since they result in the alteration of so many cellular functions (Glaser et al., 1973; Luri et al., 1975; Glembotski et al., 1981; Goelz and Cronan, 1982; Huss and Glaser, 1983). A 34-kD protein factor was reported to increase the thermola bility of the AK from the *adk*-6 mutant of *E. coli* (Huss and Glaser, 1983), which was one of the reasons we chose to look at this *adk* mutation first. The *adk*-6 mutation also resulted in a significant structural change in AK as manifested by the change in mobility on SDS-polyacrylamide gels and its thermolability.

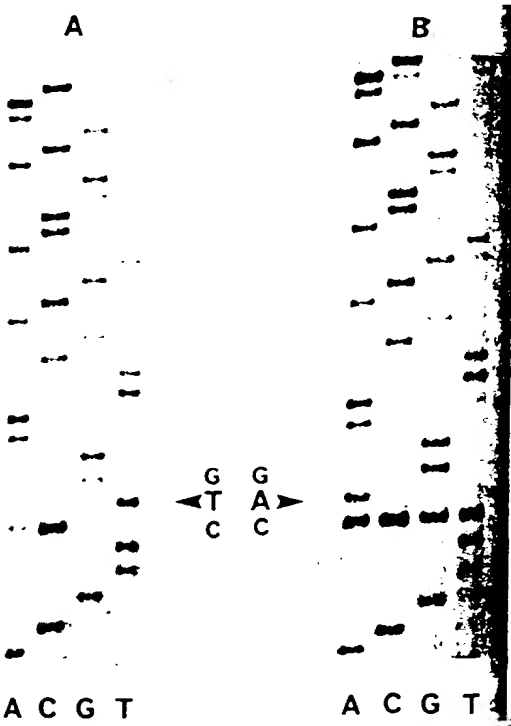

Fig. 3. Sequencing of the point mutation in the *adk*-6 gene. Both the *adk*$^+$ (on pLP101) and the *adk*-6 (on pLP202) genes were sequenced with five oligo primers which were based on the sequence published for the *adk*$^+$ gene (Brune et al., 1985). Three primers were made to sequence from the 5′ end of the gene (5′-CTCGCCATTAACCG, 5′-AGGCTCAGTTCATC and 5′-TTCCTGTTGGACGG) and two primers to sequence from the 3′ end of the gene (5′-CCGGCCTGAGATTGC and 5′-CTTCAGCAACCGGC). (Panel A) The nucleotide sequence of the *adk*$^+$ gene, CTG codes for leucine. (Panel B) A point mutation in the *adk*-6 gene, CAG codes for glutamine.

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(*1*) In this study the *adk*-6 gene has been cloned and the mutation was shown to be a single bp change to give an aa substitution of Leu-107 to Gln-107.

(*2*) Several aspects of gene homogenotization were taken into consideration in the cloning of the *adk*-6 gene. (*i*) The *recA* protein is required (Clark, 1973) and increased levels of the *recA* protein may increase the frequency of homologous recombination (Neidhardt, 1987). (*ii*) The copy-number of the plasmid is important because the recombined plasmid has to segregate from the nonrecombined ones. The lower the copy number, the easier the segregation of individual plasmid from each other (Meacock and Cohen, 1980). (*iii*) Agents such as MC will induce genetic recombination (Kenyon and Walker, 1980; Chattoraj et al., 1984). It should be noted that treatment with MC and subsequent SOS induction slightly increases the possibility of acquiring new mutations in addition to transfer of existing mutations. However, a high efficiency of gene homogenotization minimizes the possibility of isolating newly generated mutations. (*iv*) The size of the DNA insert is also an important concern; the bigger the insert, the more the chance for recombination to occur as in the case of F′ factors (Low, 1973).

(*3*) To increase the frequency of gene homogenotization a multi-copy plasmid carrying the *recA*$^+$ gene was introduced into the *recA*1, *adk*-6 mutant (KG10), and the strain was grown in the presence of MC to select for, as well as to induce, the *recA*$^+$ gene (Kenyon and Walker, 1980). A low-copy-number plasmid carrying a rather large insert with the *adk*$^+$ gene was then introduced into this strain. After recombination, the plasmid carrying the *recA*$^+$ gene was easily lost by growing the cells without MC. By this procedure a 15% efficiency of gene homogenotization was obtained for cloning the *adk*-6 gene. Similar efficiencies were also obtained in cloning two other mutant *adk* genes (not shown).

ACKNOWLEDGEMENT

We thank Douglas Au and Robert Gennis for generously providing the Mucts lysogen with the RP4 plasmid, Stanley Bower for helpful discussions, and Jean H. Lewis for excellent preparation of the manuscript. This work was supported by Department of Energy Grant DEFG02-87ER13710.

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APPENDIX E

In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988)

of Law will be entered on the same date herewith.

ORDER AND JUDGMENT

In accordance with the Findings of Fact and Conclusions of Law entered on the same date herewith,

IT IS HEREBY ORDERED AND ADJUDGED, as follows:

1. The Nolan patent (No. 4,506,189), issued on March 19, 1985, is a valid patent.

2. By the manufacture, production, sale and distribution of its SAF-T-COTE fluorescent lamp, Trojan has infringed the Nolan patent.

3. By virtue of this infringement, Shat-R-Shield is entitled to injunctive relief. Trojan shall immediately cease and desist from the manufacture, production, sale and distribution of the SAF-T-COTE fluorescent lamp.

4. Trojan shall recall all the SAF-T-COTE fluorescent lamps sold to and still in the possession of its customers.

5. The Court having determined that Trojan's infringement was not willful and wanton, Shat-R-Shield is not entitled to treble damages.

6. Shat-R-Shield shall have no accounting for monetary damages.

7. The Court having found that this is not an exceptional case, Shat-R-Shield is not entitled to its attorney's fees.

8. All claims having been resolved as to all parties herein, this action is now DISMISSED and STRICKEN from the docket.

9. There being no just reason for delay, this is a FINAL and APPEALABLE Order and Judgment.

Court of Appeals, Federal Circuit

In re Wands

No. 87-1454

Decided September 30, 1988

PATENTS

1. Patentability/Validity — Adequacy of disclosure (§115.12)

Data disclosed in application for immunoassay method patent, which shows that applicants screened nine of 143 cell lines developed for production of antibody necessary to practice invention, stored remainder of said cell lines, and found that four out of nine cell lines screened produced antibody falling within limitation of claims, were erroneously interpreted by Board of Patent Appeals and Interferences as failing to meet disclosure requirements of 35 USC 112, since board's characterization of stored cell lines as "failures" demonstrating unreliability of applicants' methods was improper in view of fact that such unscreened cell lines prove nothing concerning probability of success of person skilled in art attempting to obtain requisite antibodies using applicants' methods.

2. Patentability/Validity — Adequacy of disclosure (§115.12)

Disclosure in application for immunoassay method patent does not fail to meet enablement requirement of 35 USC 112 by requiring "undue experimentation," even though production of monoclonal antibodies necessary to practice invention first requires production and screening of numerous antibody producing cells or "hybridomas," since practitioners of art are prepared to screen negative hybridomas in order to find those that produce desired antibodies, since in monoclonal antibody art one "experiment" is not simply screening of one hybridoma but rather is entire attempt to make desired antibody, and since record indicates that amount of effort needed to obtain desired antibodies is not excessive, in view of applicants' success in each attempt to produce antibody that satisfied all claim limitations.

Appeal from decision of Patent and Trademark Office, Board of Patent Appeals and Interferences.

Application for patent of Jack R. Wands, Vincent R. Zurawski, Jr., and Hubert J. P. Schoemaker, serial number 188,735. From decision of Board of Patent Appeals and Interferences affirming rejection of application, applicants appeal. Reversed; Newman, J., concurring in part and dissenting in part in separate opinion.

Jorge A. Goldstein, of Saidman, Sterne, Kessler & Goldstein (Henry N. Wixon, with them on brief), Washington, D.C., for appellant.

John H. Raubitschek, associate solicitor (Joseph F. Nakamura and Fred E. McKelvey, with him on brief), PTO, for appellee.

Before Smith, Newman, and Bissell, circuit judges.

Smith, J.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences (board) affirming the rejection of all remaining claims in appellant's application for a patent, serial No. 188,735, entitled "Immunoassay Utilizing Monoclonal High Affinity IgM

Antibodies," which was filed September 19, 1980.[1] The rejection under 35 U.S.C. §112, first paragraph, is based on the grounds that appellant's written specification would not enable a person skilled in the art to make the monoclonal antibodies that are needed to practice the claimed invention without undue experimentation. We reverse.

I. *Issue*

The only issue on appeal is whether the board erred, as a matter of law, by sustaining the examiner's rejection for lack of enablement under 35 U.S.C. §112, first paragraph, of all remaining claims in appellants' patent application, serial No. 188,735.

II. *Background*

A. *The Art.*

The claimed invention involves immunoassay methods for the detection of hepatitis B surface antigen by using high-affinity monoclonal antibodies of the IgM isotype. *Antibodies* are a class of proteins (immunoglobulins) that help defend the body against invaders such as viruses and bacteria. An antibody has the potential to bind tightly to another molecule, which molecule is called an antigen. The body has the ability to make millions of different antibodies that bind to different antigens. However, it is only after exposure of an antigen that a complicated *immune response* leads to the production of antibodies against that antigen. For example, on the surface of hepatitis B virus particles there is a large protein called *hepatitis B surface antigen* (HBsAg). As its name implies, it is capable of serving as an antigen. During a hepatitis B infection (or when purified HBsAg is injected experimentally), the body begins to make antibodies that bind tightly and specifically to HBsAg. Such antibodies can be used as reagents for sensitive diagnostic tests (*e.g.*, to detect hepatitis B virus in blood and other tissues, a purpose of the claimed invention). A method for detecting or measuring antigens by using antibodies as reagents is called an *immunoassay*.

Normally, many different antibodies are produced against each antigen. One reason for this diversity is that different antibodies are produced that bind to different regions (determinants) of a large antigen molecule such as HBsAg. In addition, different antibodies may be produced that bind to the same determinant. These usually differ in the tightness with which they bind to the determinant. *Affinity* is a quantitative measure of the strength of antibody-antigen binding. Usually an antibody with a higher affinity for an antigen will be more useful for immunological diagnostic tests than one with a lower affinity. Another source of heterogeneity is that there are several immunoglobulin classes or *isotypes*. Immunoglobulin G (IgG) is the most common isotype in serum. Another isotype, immunoglobulin M (IgM), is prominent early in the immune response. IgM molecules are larger than IgG molecules, and have 10 antigen-binding sites instead of the 2 that are present in IgG. Most immunoassay methods use IgG, but the claimed invention uses only IgM antibodies.

For commercial applications there are many disadvantages to using antibodies from serum. Serum contains a complex mixture of antibodies against the antigen of interest within a much larger pool of antibodies directed at other antigens. There are available only in a limited supply that ends when the donor dies. The goal of monoclonal antibody technology is to produce an unlimited supply of a single purified antibody.

The blood cells that make antibodies are *lymphocytes*. Each lymphocyte makes only one kind of antibody. During an immune response, lymphocytes exposed to their particular antigen divide and mature. Each produces a *clone* of identical daughter cells, all of which secrete the same antibody. Clones of lymphocytes, all derived from a single lymphocyte, could provide a source of a single homogeneous antibody. However, lymphocytes do not survive for long outside of the body in cell culture.

Hybridoma technology provides a way to obtain large numbers of cells that all produce the same antibody. This method takes advantage of the properties of *myeloma* cells derived from a tumor of the immune system. The cancerous myeloma cells can divide indefinitely in vitro. They also have the potential ability to secrete antibodies. By appropriate experimental manipulations, a myeloma cell can be made to fuse with a lymphocyte to produce a single hybrid cell (hence, a hybridoma) that contains the genetic material of both cells. The hybridoma secretes the same antibody that was made by its parent lymphocyte, but acquires the capability of the myeloma cell to divide and grow indefinitely in cell culture. Antibodies produced by a clone of hybridoma cells (i.e., by hybridoma

[1] *In re Wands*, Appeal No. 673-76 (Bd. Pat. App. & Int. Dec. 30, 1986).

cells that are all progeny of a single cell) are called monoclonal antibodies.[2]

B. *The Claimed Invention.*

The claimed invention involves methods for the immunoassay of HBsAg by using high-affinity monoclonal IgM antibodies. Jack R. Wands and Vincent R. Zurawski, Jr., two of the three coinventors of the present application, disclosed methods for producing monoclonal antibodies against HBsAg in United States patent No. 4,271,145 (the '145 patent), entitled "Process for Producing Antibodies to Hepatitis Virus and Cell Lines Therefor," which patent issued on June 2, 1981. The '145 patent is incorporated by reference into the application on appeal. The specification of the '145 patent teaches a procedure for immunizing mice against HBsAg, and the use of lymphocytes from these mice to produce hybridomas that secrete monoclonal antibodies specific for HBsAg. The '145 patent discloses that this procedure yields both IgG and IgM antibodies with high-affinity binding to HBsAg. For the stated purpose of complying with the best mode requirement of 35 U.S.C. §112, first paragraph, a hybridoma cell line that secretes IgM antibodies against HBsAg (the 1F8 cell line) was deposited at the American Type Culture Collection, a recognized cell depository, and became available to the public when the '145 patent issued.

The application on appeal claims methods for immunoassay of HBsAg using monoclonal antibodies such as those described in the '145 patent. Most immunoassay methods have used monoclonal antibodies of the IgG isotype. IgM antibodies were disfavored in the prior art because of their sensitivity to reducing agents and their tendency to self-aggregate and precipitate. Appellants found that their monoclonal IgM antibodies could be used for immunoassay of HbsAg with unexpectedly high sensitivity and specificity. Claims 1, 3, 7, 8, 14, and 15 are drawn to methods for the immunoassay of HBsAg using high-affinity IgM monoclonal antibodies. Claims 19 and 25–27 are for chemically modified (*e.g.*, radioactively labeled) monoclonal IgM antibodies used in the assays. The broadest method claim reads:

> 1. An immunoassay method utilizing an antibody to assay for a substance comprising hepatitis B-surface antigen (HBsAg) determinants which comprises the steps of:
>
> contacting a test sample containing said substance comprising HBsAg determinants with said antibody; and
>
> determining the presence of said substance in said sample;
>
> wherein said antibody is a monoclonal high affinity IgM antibody having a binding affinity constant for said HBsAg determinants of at least 10^9 M^{-1}.

Certain claims were rejected under 35 U.S.C. §103; these rejections have not been appealed. Remaining claims 1, 3, 7, 8, 14, 15, 19, and 25–27 were rejected under 35 U.S.C. §112, first paragraph, on the grounds that the disclosure would not enable a person skilled in the art to make and use the invention without undue experimentation. The rejection is directed solely to whether the specification enables one skilled in the art to make the monoclonal antibodies that are needed to practice the invention. The position of the PTO is that data presented by Wands show that the production of high-affinity IgM anti-HBsAg antibodies is unpredictable and unreliable, so that it would require undue experimentation for one skilled in the art to make the antibodies.

III. *Analysis*

A. *Enablement by Deposit of Micro-organisms and Cell Lines.*

The first paragraph of 35 U.S.C. §112 requires that the specification of a patent must enable a person skilled in the art to make and use the claimed invention. "Patents * * * are written to enable those skilled in the art to practice the invention."[3] A patent need not disclose what is well known in the art.[4] Although we review underlying facts found by the board under a "clearly erroneous" standard,[5] we review enablement as a question of law.[6]

Where an invention depends on the use of living materials such as microorganisms or

[2] For a concise description of monoclonal antibodies and their use in immunoassay see *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1368–71, 231 USPQ 81, 82–83 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987).

[3] *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

[4] *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

[5] *Coleman v. Dines*, 754 F.2d 353, 356, 224 USPQ 857, 859 (Fed. Cir. 1985).

[6] *Moleculon Research Corp. v. CBS, Inc.*, 793 F.2d 1261, 1268, 229 USPQ 805, 810 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 875 (1987); *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960 n.6, 220 USPQ 592, 599 n.6 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 835 [225 USPQ 232] (1984).

cultured cells, it may be impossible to enable the public to make the invention (i.e., to obtain these living materials) solely by means of a written disclosure. One means that has been developed for complying with the enablement requirement is to deposit the living materials in cell depositories which will distribute samples to the public who wish to practice the invention after the patent issues.[7] Administrative guidelines and judicial decisions have clarified the conditions under which a deposit of organisms can satisfy the requirements of section 112.[8] A deposit has been held necessary for enablement where the starting materials (i.e., the living cells used to practice the invention, or cells from which the required cells can be produced) are not readily available to the public.[9] Even when starting materials are available, a deposit has been necessary where it would require undue experimentation to make the cells of the invention from the starting materials.[10]

In addition to satisfying the enablement requirement, deposit of organisms also can be used to establish the filing date of the application as the prima facie date of invention,[11] and to satisfy the requirement under 35 U.S.C. §114 that the PTO be guaranteed access to the invention during pendency of the application.[12] Although a deposit may serve these purposes, we recognized, in *In re Lundak*,[13] that these purposes, nevertheless, may be met in ways other than by making a deposit.

A deposit also may satisfy the best mode requirement of section 112, first paragraph, and it is for this reason that the 1F8 hybridoma was deposited in connection with the '145 patent and the current application. Wands does not challenge the statements by the examiner to the effect that, although the deposited 1F8 line enables the public to perform immunoassays with antibodies produced by that single hybridoma, the deposit does not enable the generic claims that are on appeal. The examiner rejected the claims on the grounds that the written disclosure was not enabling and that the deposit was inadequate. Since we hold that the written disclosure fully enables the claimed invention, we need not reach the question of the adequacy of deposits.

B. *Undue Experimentation.*

Although inventions involving microorganisms or other living cells often can be enabled by a deposit,[14] a deposit is not always necessary to satisfy the enablement requirement.[15] No deposit is necessary if the biological organisms can be obtained from readily available sources or derived from readily available starting materials through routine screening that does not require undue experimentation.[16] Whether the specification in an application involving living cells (here, hybridomas) is enabled without a deposit must be decided on the facts of the particular case.[17]

Appellants contend that their written specification fully enables the practice of

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[7] *In re Argoudelis*, 434 F.2d 1390, 1392-93, 168 USPQ 99, 101-02 (CCPA 1970).

[8] *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985); *Feldman v. Aunstrup*, 517 F.2d 1351, 186 USPQ 108 (CCPA 1975), *cert. denied*, 424 U.S. 912 [188 USPQ 720] (1976); Manual of Patent Examining Procedure (MPEP) 608.01 (p)(C) (5th ed. 1983, rev. 1987). *See generally* Hampar, *Patenting of Recombinant DNA Technology: The Deposit Requirement*, 67 J. Pat. Trademark Off. Soc'y 569 (1985).

[9] *In re Jackson*, 217 USPQ 804, 807-08 (Bd. App. 1982) (strains of a newly discovered species of bacteria isolated from nature); *Feldman*, 517 F.2d 1351, 186 USPQ 108 (uncommon fungus isolated from nature); *In re Argoudelis*, 434 F.2d at 1392, 168 USPQ at 102 (novel strain of antibiotic-producing microorganism isolated from nature); *In re Kropp*, 143 USPQ 148, 152 (Bd. App. 1959) (newly discovered microorganism isolated from soil).

[10] *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (genetically engineered bacteria where the specification provided insufficient information about the amount of time and effort required); *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (unique cell line produced from another cell line by mutagenesis).

[11] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95-96; *In re Feldman*, 517 F.2d at 1355, 186 USPQ at 113; *In re Argoudelis*, 434 F.2d at 1394-96, 168 USPQ at 103-04 (Baldwin, J. concurring).

[12] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95-96; *In re Feldman*, 517 F.2d at 1354, 186 USPQ at 112.

[13] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95-96.

[14] *In re Argoudelis*, 434 F.2d at 1393, 168 USPQ at 102.

[15] *Tabuchi v. Nubel*, 559 F.2d 1183, 194 USPQ 521 (CCPA 1977).

[16] *Id.* at 1186-87, 194 USPQ at 525; *Merck & Co. v. Chase Chem. Co.*, 273 F.Supp. 68, 77, 155 USPQ 139, 146 (D.N.J. 1967); *Guaranty Trust Co. v. Union Solvents Corp.*, 54 F.2d 400, 403-06, 12 USPQ 47, 50-53 (D. Del. 1931), *aff'd.* of F.2d 1041, 15 USPQ 237 (3d Cir. 1932), *cert. denied*, 288 U.S. 614 (1933); MPEP 608.01(p)(C) ("No problem exists when the microorganisms used are known and readily available to the public.").

[17] *In re Jackson*, 217 USPQ at 807; *see In re Metcalfe*, 410 F.2d 1378, 1382, 161 USPQ 789, 792 (CCPA 1969).

their claimed invention because the monoclonal antibodies needed to perform the immunoassays can be made from readily available starting materials using methods that are well known in the monoclonal antibody art. Wands states that application of these methods to make high-affinity IgM anti-HBsAg antibodies requires only routine screening, and that does not amount to undue experimentation. There is no challenge to their contention that the starting materials (i.e., mice, HBsAg antigen, and myeloma cells) are available to the public. The PTO concedes that the methods used to prepare hybridomas and to screen them for high-affinity IgM antibodies against HBsAg were either well known in the monoclonal antibody art or adequately disclosed in the '145 patent and in the current application. This is consistent with this court's recognition with respect to another patent application that methods for obtaining and screening monoclonal antibodies were well known in 1980.[18] The sole issue is whether, in this particular case, it would require undue experimentation to produce high-affinity IgM monoclonal antibodies.

Enablement is not precluded by the necessity for some experimentation such as routine screening.[19] However, experimentation needed to practice the invention must not be undue experimentation.[20] "the key word is 'undue,' not 'experimentation.' "[21]

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. Ansul Co. v. Uniroyal, Inc. [448 F.2d 872, 878–79; 169 USPQ 759, 762–63 (2d Cir. 1971), *cert. denied*, 404 U.S. 1018 [172 USPQ 257] (1972)]. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed * * *.[22]

The term "undue experimentation" does not appear in the statute, but it is well established that enablement requires that the specification teach those in the art to make and use the invention without undue experimentation.[23] Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations. The board concluded that undue experimentation would be needed to practice the invention on the basis of experimental data presented by Wands. These data are not in dispute. However, Wands and the board disagree strongly on the conclusion that should be drawn from that data.

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*.[24] They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.[25]

In order to understand whether the rejection was proper, it is necessary to discuss further the methods for making specific monoclonal antibodies. The first step for making monoclonal antibodies is to immunize an animal. The '145 patent provides a detailed description of procedures for immunizing a specific strain of mice against HBsAg. Next the spleen, an organ rich in lymphocytes, is removed and the lymphocytes are separated from the other spleen cells. The lymphocytes are mixed with myeloma cells, and the mixture is treated to cause a few of the cells to fuse with each other. Hybridoma cells that secrete the desired antibodies then must be isolated from the enormous number of other cells in the mixture. This is done through a series of screening procedures.

The first step is to separate the hybridoma cells from unfused lymphocytes and myeloma cells. The cells are cultured in a medi-

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[18] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94.

[19] *Id.*; *Atlas Powder Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984); *In re Angstadt*, 537 F.2d at 502–504, 190 USPQ at 218; *In re Geerdes*, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); *Mineral Separation, Ltd. v. Hyde*, 242 U.S. 261, 270–71 (1916).

[20] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *W.L. Gore*, 721 F.2d at 1557, 220 USPQ at 316; *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977) (Miller, J., concurring).

[21] *In re Angstadt*, 537 F.2d at 504, 190 USPQ at 219.

[22] *In re Jackson*, 217 USPQ at 807.

[23] *See Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *Atlas Powder*, 750 F.2d at 1576, 224 USPQ at 413.

[24] *Ex parte Forman*, 230 USPQ at 547.

[25] *Id.*, *see In re Colianni*, 561 F.2d at 224, 195 USPQ at 153 (Miller, J., concurring); *In re Rainer*, 347 F.2d 574, 577, 146 USPQ 218, 221 (CCPA 1965).

um in which all the lymphocytes and myeloma cells die, and only the hybridoma cells survive. The next step is to isolate and clone hybridomas that make antibodies that bind to the antigen of interest. Single hybridoma cells are placed in separate chambers and are allowed to grow and divide. After there are enough cells in the clone to produce sufficient quantities of antibody to analyze, the antibody is assayed to determine whether it binds to the antigen. Generally, antibodies from many clones do not bind the antigen, and these clones are discarded. However, by screening enough clones (often hundreds at a time), hybridomas may be found that secrete antibodies against the antigen of interest.

Wands used a commercially available radioimmunoassay kit to screen clones for cells that produce antibodies directed against HBsAg. In this assay the amount of radioactivity bound gives some indication of the strength of the antibody-antigen binding, but does not yield a numerical affinity constant, which must be measured using the more laborious Scatchard analysis. In order to determine which anti-HBsAg antibodies satisfy all of the limitations of appellants' claims, the antibodies require further screening to select those which have an IgM isotype and have a binding affinity constant of at least 10^9 M^{-1}.[36] The PTO does not question that the screening techniques used by Wands were well known in the monoclonal antibody art.

During prosecution Wands submitted a declaration under 37 C.F.R §1.132 providing information about all of the hybridomas that appellants had produced before filing the patent application. The first four fusions were unsuccessful and produced no hybridomas. The next six fusion experiments all produced hybridomas that made antibodies specific for HBsAg. Antibodies that bound at least 10,000 cpm in the commercial radioimmunoassay were classified as "high binders." Using this criterion, 143 high-binding hybridomas were obtained. In the declaration, Wands stated that [37]

> It is generally accepted in the art that, among those antibodies which are binders with 50,000 cpm or higher, there is a very high likelihood that high affinity (Ka [greater than] 10^9 M^{-1}) antibodies will be found. However, high affinity antibodies can also be found among high binders of between 10,000 and 50,000, as is clearly demonstrated in the Table.

The PTO has not challenged this statement.

The declaration stated that a few of the high-binding monoclonal antibodies from two fusions were chosen for further screening. The remainder of the antibodies and the hybridomas that produced them were saved by freezing. Only nine antibodies were subjected to further analysis. Four (three from one fusion and one from another fusion) fell within the claims, that is, were IgM antibodies and had a binding affinity constant of at least 10^9 M^{-1}. Of the remaining five antibodies, three were found to be IgG, while the other two were IgM for which the affinity constants were not measured (although both showed binding well above 50,000 cpm).

Apparently none of the frozen cell lines received any further analysis. The declaration explains that after useful high-affinity IgM monoclonal antibodies to HBsAg had been found, it was considered unnecessary to return to the stored antibodies to screen for more IgMs. Wands says that the existence of the stored hybridomas was disclosed to the PTO to comply with the requirement under 37 C.F.R. §1.56 that applicants fully disclose all of their relevant data, and not just favorable results.[38] How these stored hybridomas are viewed is central to the positions of the parties.

The position of the board emphasizes the fact that since the stored cell lines were not completely tested, there is no proof that any of them are IgM antibodies with a binding affinity constant of at least 10^9 M^{-1}. Thus, only 4 out of 143 hybridomas, or 2.8 percent, were *proved* to fall within the claims. Furthermore, antibodies that were proved to be high-affinity IgM came from only 2 of 10 fusion experiments. These statistics are viewed by the board as evidence that appellants' methods were not predictable or reproducible. The board concludes that Wands' low rate of demonstrated success shows that a person skilled in the art would have to

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[36] The examiner, the board, and Wands all point out that, technically, the strength of antibody-HBsAg binding is measured as *avidity*, which takes into account multiple determinants on the HBsAg molecule, rather than affinity. Nevertheless, despite this correction, all parties then continued to use the term "affinity." We will use the terminology of the parties. Following the usage of the parties, we will also use the term "high-affinity" as essentially synonymous with "having a binding affinity constant of at least 10^9 M^{-1}."

[37] A table in the declaration presented the binding data for antibodies from every cell line. Values ranged from 13,867 to 125,204 cpm, and a substantial proportion of the antibodies showed binding greater than 50,000 cpm. In confirmation of Dr. Wand's statement, two antibodies with binding less than 25,000 cpm were found to have affinity constants greater than 10^9 M^{-1}.

[38] *See Rohm & Haas Co. v. Crystal Chem. Co.*, 722 F.2d 1556, 220 USQ 98 (Fed. Cir. 1983).

engage in undue experimentation in order to make antibodies that fall within the claims.

Wands views the data quite differently. Only nine hybridomas were actually analyzed beyond the initial screening for HBsAg binding. Of these, four produced antibodies that fell within the claims, a respectable 44 percent rate of success. (Furthermore, since the two additional IgM antibodies for which the affinity constants were never measured showed binding in excess of 50,000 cpm, it is likely that these also fall within the claims.) Wands argues that the remaining 134 unanalyzed, stored cell lines should not be written off as failures. Instead, if anything, they represent partial success. Each of the stored hybridomas had been shown to produce a high-binding antibody specific for HBsAg. Many of these antibodies showed binding above 50,000 cpm and are thus highly likely to have a binding affinity constant of at least 10^9 M^{-1}. Extrapolating from the nine hybridomas that were screened for isotype (and from what is well known in the monoclonal antibody art about isotype frequency), it is reasonable to assume that the stored cells include some that produce IgM. Thus, if the 134 incompletely analyzed cell lines are considered at all, they provide some support (albeit without rigorous proof) to the view that hybridomas falling within the claims are not so rare that undue experimentation would be needed to make them.

The first four fusion attempts were failures, while high-binding antibodies were produced in the next six fusions. Appellants contend that the initial failures occurred because they had not yet learned to fuse cells successfully. Once they became skilled in the art, they invariably obtained numerous hybridomas that made high-binding antibodies against HBsAg and, in each fusion where they determined isotype and binding affinity they obtained hybridomas that fell within the claims.

Wands also submitted a second declaration under 37 C.F.R. §1.132 stating that after the patent application was submitted they performed an eleventh fusion experiment and obtained another hybridoma that made a high-affinity IgM anti-HBsAg antibody. No information was provided about the number of clones screened in that experiment. The board determined that, because there was no indication as to the number of hybridomas screened, this declaration had very little value. While we agree that it would have been preferable if Wands had included this information, the declaration does show that when appellants repeated their procedures they again obtained a hybridoma that produced an antibody that fit all of the limitations of their claims.

[1] We conclude that the board's interpretation of the data is erroneous. It is strained and unduly harsh to classify the stored cell lines (each of which was proved to make high-binding antibodies against HBsAg) as failures demonstrating that Wands' methods are unpredictable or unreliable.[*] At worst, they prove nothing at all about the probability of success, and merely show that appellants were prudent in not discarding cells that might someday prove useful. At best, they show that high-binding antibodies, the starting materials for IgM screening and Scatchard analysis, can be produced in large numbers. The PTO's position leads to the absurd conclusion that the more hybridomas an applicant makes and saves without testing the less predictable the applicant's results become. Furthermore, Wands' explanation that the first four attempts at cell fusion failed only because they had not yet learned to perform fusions properly is reasonable in view of the fact that the next six fusions were all successful. The record indicates that cell fusion is a technique that is well known to those of ordinary skill in the monoclonal antibody art, and there has been no claim that the fusion step should be more difficult or unreliable where the antigen is HBsAg than it would be for other antigens.

[2] When Wands' data is interpreted in a reasonable manner, analysis considering the factors enumerated in *Ex parte Forman* leads to the conclusion that undue experimentation would not be required to practice the invention. Wands' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known.

The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. No evidence was presented by either party on how many hybridomas would be viewed by those in the art as requiring undue experimentation to screen. However, it seems unlikely that un-

[*] Even if we were to accept the PTO's 2.8% success rate, we would not be required to reach a conclusion of undue experimentation. Such a determination must be made in view of the circumstances of each case and cannot be made solely by reference to a particular numerical cutoff.

due experimentation would be defined in terms of the number of hybridomas that were never screened. Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics. Wands carried out this entire procedure three times, and was successful each time in making at least one antibody that satisfied all of the claim limitations. Reasonably interpreted, Wands' record indicates that, in the production of high-affinity IgM antibodies against HBsAG, the amount of effort needed to obtain such antibodies is not excessive. Wands' evidence thus effectively rebuts the examiner's challenge to the enablement of their disclosure.[10]

IV. *Conclusion*

Considering all of the factors, we conclude that it would not require undue experimentation to obtain antibodies needed to practice the claimed invention. Accordingly, the rejection of Wands' claims for lack of enablement under 35 U.S.C. §112, first paragraph, is reversed.

REVERSED

Newman, J., concurring in part, dissenting in part.

A

I concur in the court's holding that additional samples of hybridoma cell lines that produce these high-affinity IgM monoclonal antibodies need not be deposited. This invention, as described by Wands, is not a selection of a few rare cells from many possible cells. To the contrary, Wands states that all monoclonally produced IgM antibodies to hepatitis B surface antigen have the desired high avidity and other favorable properties, and that all are readily preparable by now-standard techniques.

Wands states that his United States Patent No. 4,271,145 describes fully operable techniques, and is distinguished from his first four failed experiments that are referred to in the Rule 132 affidavit. Wands argues that these biotechnological mechanisms are relatively well understood and that the preparations can be routinely duplicated by those of skill in this art, as in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987). I agree that it is not necessary that there be a deposit of multiple exemplars of a cell system that is readily reproduced by known, specifically identified techniques.

B

I would affirm the board's holding that Wands has not complied with 35 U.S.C. §112, first paragraph, in that he has not provided data sufficient to support the breadth of his generic claims. Wands' claims on appeal include the following:

> 19. Monoclonal high affinity IgM antibodies immunoreactive with HBsAg determinants, wherein said antibodies are coupled to an insoluble solid phase, and wherein the binding affinity constant of said antibodies for said HBsAg determinants is at least 10^9 M^{-1}.
>
> 26. Monoclonal high affinity IgM antibodies immunoreactive with hepatitis B surface antigen.

Wands states that he obtained 143 "high binding monoclonal antibodies of the right specificity" in the successful fusions; although he does not state how they were determined to be high binding or of the right specificity, for Wands also states that only nine of these 143 were tested.

Of these nine, four (three from one fusion and one from another fusion) were found to have the claimed high affinity and to be of the IgM isotype. Wands states that the other five were either of a different isotype or their affinities were not determined. (This latter statement also appears to contradict his statement that all 143 were "high binding".)

Wands argues that a "success rate of four out of nine", or 44.4%, is sufficient to support claims to the entire class. The Commissioner deems the success rate to be four out of 143, or 2.8%; to which Wands responds with statistical analysis as to how unlikely it is that Wands selected the only four out of 143 that worked. Wands did not, however, prove the right point. The question is whether Wands, by testing nine out of 143 (the Commissioner points out that the randomness of the sample was not established), and finding that four out of the nine had the desired properties, has provided sufficient experimental support for the breadth of the requested claims, in the context that "experi-

[10] *In re Strahilevitz*, 668 F.2d 1229, 1232, 212 USPQ 561, 563 (CCPA 1982).

ments in genetic engineering produce, at best, unpredictable results", quoting from *Ex parte Forman*, 230 USPQ 546, 547 (Bd.Pat.App. and Int. 1986).

The premise of the patent system is that an inventor, having taught the world something it didn't know, is encouraged to make the product available for public and commercial benefit, by governmental grant of the right to exclude others from practice of that which the inventor has disclosed. The boundary defining the excludable subject matter must be carefully set: it must protect the inventor, so that commercial development is encouraged; but the claims must be commensurate with the inventor's contribution. Thus the specification and claims must meet the requirements of 35 U.S.C. §112. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 23-24 (CCPA 1970).

As the science of biotechnology matures the need for special accommodation, such as the deposit of cell lines or microorganisms, may diminish; but there remains the body of law and practice on the need for sufficient disclosure, including experimental data when appropriate, that reasonably support the scope of the requested claims. That law relates to the sufficiency of the description of the claimed invention, and if not satisfied by deposit, must independently meet the requirements of Section 112.

Wands is not claiming a particular, specified IgM antibody. He is claiming all such monoclonal antibodies in assay for hepatitis B surface antigen, based on his teaching that such antibodies have uniformly reproducible high avidity, free of the known disadvantages of IgM antibodies such as tendency to precipitate or aggregate. It is incumbent upon Wands to provide reasonable support for the proposed breadth of his claims. I agree with the Commissioner that four exemplars shown to have the desired properties, out of the 143, do not provide adequate support.

Wands argues that the law should not be "harsher" where routine experiments take a long time. However, what Wands is requesting is that the law be less harsh. As illustrated in extensive precedent on the question of how much experimentation is "undue", each case must be determined on its own facts. *See, e.g., W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1557, 220 USPQ 303, 316 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 218 (CCPA 1976); *In re Cook*, 439 F.2d 730, 734-35, 169 USPQ 298, 302-03 (CCPA 1971).

The various criteria to be considered in determining whether undue experimentation is required are discussed in, for example, *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971); *In re Rainer*, 347 F.2d 574, 146 USPQ 218 (CCPA 1965); *Ex parte Forman*, 230 USPQ at 547. Wands must provide sufficient data or authority to show that his results are reasonably predictable within the scope of the claimed generic invention, based on experiment and/or scientific theory. In my view he has not met this burden.

Patent and Trademark Office
Trademark Trial and Appeal Board

In re Johanna Farms Inc.

Serial No. 542,343

Decided June 30, 1988

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Prior adjudication — In general (§410.1501)

Trademark Trial and Appeal Board's prior decision upholding examiner's refusal to register proposed mark "La Yogurt" does not preclude registration of mark pursuant to subsequent application, since applicant, by presenting survey evidence and consumer letters regarding issue of how purchasers perceive proposed mark, has demonstrated that instant factual situation is different from situation presented in prior proceeding.

TRADEMARKS AND UNFAIR TRADE PRACTICES

2. Types of marks — Non-descriptive — Particular marks (§327.0505)

Term "La Yogurt," with "yogurt" disclaimed, is registrable, since word "yogurt" is common English generic term rather than corruption or misspelling of French word for yogurt, since examining attorney failed to meet burden of showing clear evidence of generic use of mark as whole, and since evidence of record, including survey and consumer letters to applicant, demonstrates that primary significance of "La Yogurt" to majority of relevant public is that of brand name rather than generic term.

APPENDIX F

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APPENDIX F

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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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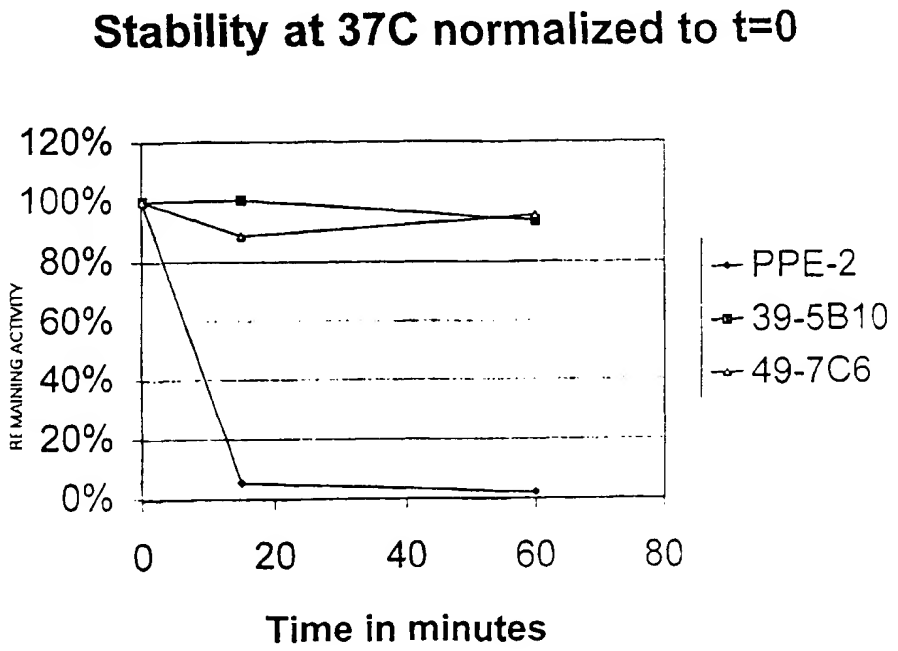
(54) Title: THERMOSTABLE LUCIFERASES AND METHODS OF PRODUCTION



(57) Abstract

Luciferase enzymes with greatly increased thermostability, e.g., at least half lives of 2 hours at 50 °C, cDNAs encoding the novel luciferases, and hosts transformed to express the luciferases, are disclosed. Methods of producing the luciferases include recursive mutagenesis. The luciferases are used in conventional methods, some employing kits.